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THE JOURNAL OF PARASITOLOGY

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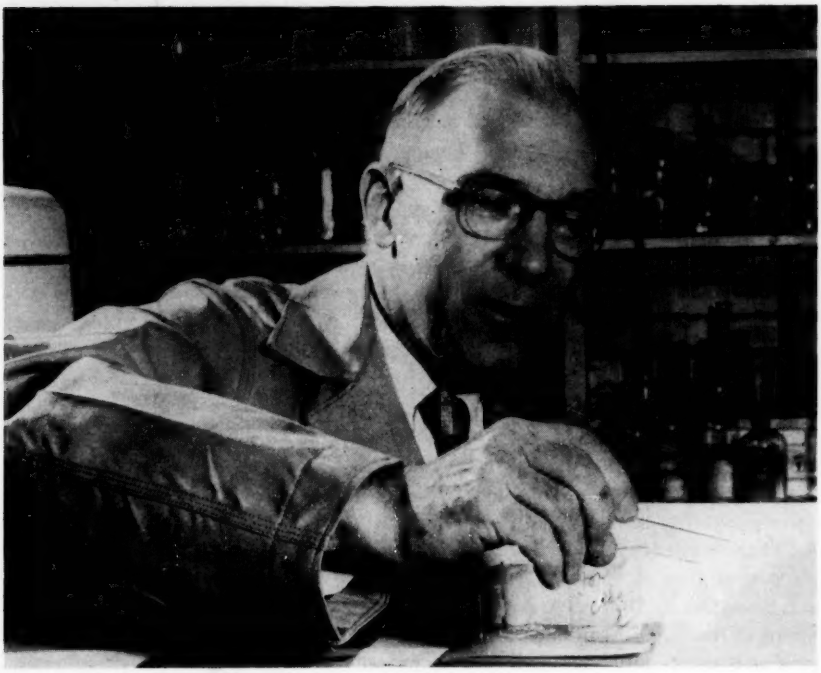
CHESTER ALBERN HERRICK
1893–1955

Death removed from our midst some months ago a creative investigator and an inspiring teacher, Professor Chester Albern Herrick, University of Wisconsin, who was approaching the crest of a distinguished career. Trained and initiated especially in helminthology, he worked primarily with nematodes for a decade before violent allergic reactions to *Ascaris* forced him from this field.



CHESTER ALBERN HERRICK

It was like the proverbial being "kicked upstairs." Helminth research was terminated and the formidable fowl coccidiosis problem attacked. From the basic behavior of *Eimeria tenella* oöcysts under incubator conditions with the chicken as host to a major look into fowl coccidiosis, professor and students warmed to their task.

Received for publication, October 22, 1956.

Factors in the severity of cecal coccidiosis, the role of glycogen and other carbohydrates, the respiration of the parasite, production of muscular fatigue and the greater resistance of heavier breeds of fowls to the effects of this organism were among the early phases investigated.

Other host-parasite relationships studied included physiological responses of the host to the parasite, the reaction of the cecal pouch to the organisms, the pH of the pouch, its metabolism, function during infection, associated bacteriology, and changes in both the cellular and the plasma portions of the blood during infection.

Brilliant as many of his researches have been, it is the concensus of his intimate associates that some of his finest contributions remain unpublished. It is their belief that he contemplated the preparation of a monograph on coccidiosis when he felt that they had made contributions sufficient to justify such an authoritative publication.

Desires to check or prevent the ravages of coccidiosis in fowls led to a trial of sulphur, only to be followed by a query on sulphur and rickets in chickens. Further "boring" into the problem with tests of ten synthetic nitrogen-sulfur compounds, known to have been effective against certain other parasitic organisms, gave evidence of "pay dirt," for three of the ten were found to give protection of chickens against experimental cecal coccidiosis!

Of the three compounds, the best one appeared to be tetraethylthiuram monosulphide which, when given in suitable dosages at the time of administration of oöcysts, resulted in the development of resistance in chickens to coccidiosis. There remained only the painstaking tests to develop a practical method of giving the oöcysts and administering the chemical to produce resistance in chickens to cecal coccidiosis.

The parasitic fauna of fish and game animals were given some attention by Professor Herrick and his advanced students, twenty-three of whom obtained the doctorate during the twenty-eight years he served at Wisconsin. In more recent years a collaborative study of the sheep stomach worm, *Haemonchus contortus,* was getting under way—two of four papers having six authors. Other investigations by Professor Herrick included quantitative studies on dog hookworm infections, effects of *Ascaris* extract on circulation and respiration, development of resistance in young rats to trypanosomes, presence of *Ornithodorus talaje* in Wisconsin homes and *Nosema* disease of bees. The published papers at the time of his passing numbered sixty-two.

Born in Kirwin, Kansas, in 1893, Herrick moved with his family to a farm in the eastern part of the state. He was graduated from Kansas State College with a B.S. degree in 1921 after serving two years as a radio technician in the Signal Corps of the U. S. Army during World War I. In 1923 he received the M.S. degree at Kansas State where with the senior author the principle of host age resistance to helminths was first established. Continuing these studies at the same institution and then at Johns Hopkins University, Herrick received the Sc.D. degree in 1925.

During the next two years he filled a summer research assignment in Alabama for the International Health Board and served at Johns Hopkins as research associate and as instructor until he went to the University of Wisconsin in 1927. His appointment, first in Zoology, was made a joint one with Veterinary Science in

1931. This arrangement proved to be highly satisfactory as he became associate professor in 1936 and professor in 1942 in both departments.

Dr. Herrick's heart and soul were in his service to the two departments espevially in devising and building research equipment for his students, in preparing material for teaching and in acting as a consultant for fox farmers. An example of the first was his design and construction of a device which enabled many to study the metabolism of several small laboratory animals at one time. As a teacher he was popular and effective. Added to his colorful personality were a commanding knowledge of his field, beautifully illustrated lectures, choice laboratory demonstrations, and exquisite plates of parasites of man and animals.

Highly regarded was his collection of parasites in which he took great pride. He was persistent in acquiring specimens from animals and from man, especially from war service returnees. Experienced parasitologists examining his collection have been amazed at the variety of the specimens and the high quality of the preparations. Their comparisons of his collection with those of other institutions more widely known in the field of parasitology resulted in the conviction that the Herrick collection was as good as any and better than most of them. Also attesting to the value of his collection and to his recognition as a teacher was his annual participation in the parasitological instruction offered at Walter Reed Hospital in Washington, D. C.

Professor Herrick worked most actively in the American Society of Parasitologists and the Midwest Conference of Parasitologists, having served on the council of the former and as president of the latter. He held membership also in the Poultry Science Association, the American Society of Zoologists, the Conference of Research Workers in Animal Diseases in North America, the American Association for the Advancement of Science, the Wisconsin Academy of Sciences, Arts and Letters, Sigma Xi, Gamma Alpha, Alpha Zeta, Gamma Sigma Delta and Phi Kappa Phi.

He is survived by his wife, Elva Mall Herrick, their two children, Raymond B. and Alberna (Mrs. D. E. Oehlerts), one grandchild, two brothers and three sisters.

Chester had an infectious mirth that made him a favorite. Around a fireplace he would radiate sublime comfort and exchange witticisms. In a group, mostly his elders, the mention of "Grandpaw" aroused laughter and recalling of humorous episodes. His mirth and ingenuity, perhaps, were the supplemental qualities that enabled him to accomplish so much with so many. His death on October 13, 1955 took from us an endeared colleague and a distinguished active parasitologist.

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SPERMATOPHORES—THE MODE OF INSEMINATION OF CHIGGERS (ACARINA: TROMBICULIDAE)*

L. J. Lipovsky,[1] G. W. Byers[2] and E. H. Kardos[3]

Although chiggers have been reared and carefully observed in the laboratory, no one has seen them in copulation. Finally, after observations by the senior author extending over 8 years, it has been found that chiggers accomplish insemination with spermatophores. This paper describes the deposition of spermatophores by the male and the behavior of the female as she is inseminated.

For the past several years, structures resembling the fruiting bodies of Mucorales-like molds were noted in chigger cultures. This resemblance was, in fact, so striking that attempts were made to culture these "molds." However, certain unique features of these structures became apparent as further observations were made. Mycelial growth could never be demonstrated. These structures were never seen in tubes containing only larvae, nymphs or females. They were commonly found in tubes containing adult male chiggers and frequently they appeared in freshly prepared culture tubes within minutes after a male was introduced. These observations suggested that the stalked bodies were not of plant origin but rather depositions by male chiggers. It is important to note that definitive observations could not be made until a reliable method was found for determining the sex of living adult chiggers and until, in the case of mixed cultures, large numbers of chiggers, identified from correlated larval skins, could be reared individually to the adult stage.

Further observation and experimentation have demonstrated that these structures deposited by males are in fact stalked spermatophores and that female chiggers can be inseminated by coming in contact in a suitable manner with these spermatophores. This process of insemination has been noted in other arthropods, some closely related to Trombiculidae. Lundblad (1929) reported the deposition of sessile, stalked, spermatophores in aquatic environments by males of *Arrhenurus* (= *Arrenurus*) and other hydracarine genera. Males were noted directing females into position over the spermatophores. André (1953) in observing *Allothrombium*, described the emission of the spermatophore by the male and the manner in which the female picked up the spermatophore. Schaller (1954) presented measurement data on spermatophores of the oribatid mite *Belba geniculosa*. Spermatophores of several types have been described from a wide variety of other arthropods, including ticks, pseudoscorpions, scorpions and some insects.

MATERIALS AND METHODS

Observations were made on several chigger species in the course of these studies, but *Trombicula* (*Eutrombicula*) *splendens* Ewing was selected for experimental

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studies because this species was available in adequate numbers from pure laboratory mass cultures. The only other American species studied was an undetermined species of *Hannemania,* which was maintained only long enough to provide several spermatophores. Observations were also made on spermatophores deposited by the following species of chiggers collected in Korea: *Trombicula* (*Leptotrombidium*) *orientalis* Schluger, *Euschöngastia kitajimai* Fukuzumi and Obata, *T.* (*L.*) *palpalis* Nagayo et al., *T.* (*L.*) *pallida* Nagayo et al. and an unidentified member of the subgenus *Leptotrombidium.* With the exception of *E. kitajimai,* all specimens were reared individually, and identifications were made from cast larval skins.

The individual rearing containers used throughout this study were 1-dram Kimble "Opticlear" plastic-stoppered vials, approximately half-filled with a mixture of plaster of Paris and powdered charcoal as a substrate. The walls were left clear so that observations could be made without removing the stoppers.

An attempt was made to standardize conditions in all experimental tubes by providing known amounts of food and essentially similar conditions of temperature and humidity. Each nymph was provided with 10 Collembola eggs and each adult with 20 Collembola eggs every second day, according to the methods of Lipovsky (1951). All chigger tubes were maintained at ambient room temperature which ranged from 66° to 85° F. The substrate of each tube was watered regularly at 4-day intervals and excess water was removed with absorbent gauze.

The sexes of all species could be distinguished by the presence of specialized setae located in the genital area of the males and their complete absence in females. It was possible to determine the sex of each adult through the glass vials in which they were maintained. Males were then combined in stender dish cultures (Lipovsky, 1953) for convenience of handling.

In making observations on the acts of spermatophore-deposition and of insemination, advantage was taken of the tendency of chiggers to frequent grooves and pits. Therefore, cuboidal pits of 2 to 3 mm were excavated in the substrate, adjacent to the glass wall of each vial. Spermatophores were most frequently deposited on the walls and floors of these pits. On rare occasions, they were deposited on smooth glass walls of culture tubes. The selective placement of spermatophores in pits thus enabled observations to be made of the males depositing spermatophores and of the females harvesting them from most favorable lateral and ventral aspects. When 1 or more spermatophores were deposited in a pit, the male was removed and a virgin female was then placed in the tube containing the spermatophores.

The criterion for fertilization was the oviposition of viable eggs from which larvae emerged.

The spermatophores used in the virgin female fertilization experiment were collected within a period of 48 hours or less after deposition.

RESULTS

An experiment designed to determine the method of fertilization of *T. splendens,* is summarized and results outlined in Text-figure 1. The experiment was initiated with 4 groups of virgin females. *Group A* females paired with males were observed 40 days. The first oviposition occurred on day 10, the last occurred on day 33. No attempt was made to determine the presence of spermatophores in these tubes. *Group B* females were maintained in isolation for 50 days, during which

time no ova were deposited. On day 50 each female was exposed to a single spermatophore and observations were extended to day 175, at which time, of the 19 surviving females, 10 had deposited ova. Of these ten, 8 laid their first eggs on days 9 to 16, one delayed until day 38, and the preoviposition period of the tenth female following exposure to a single spermatophore was 117 days. *Group C* fe-

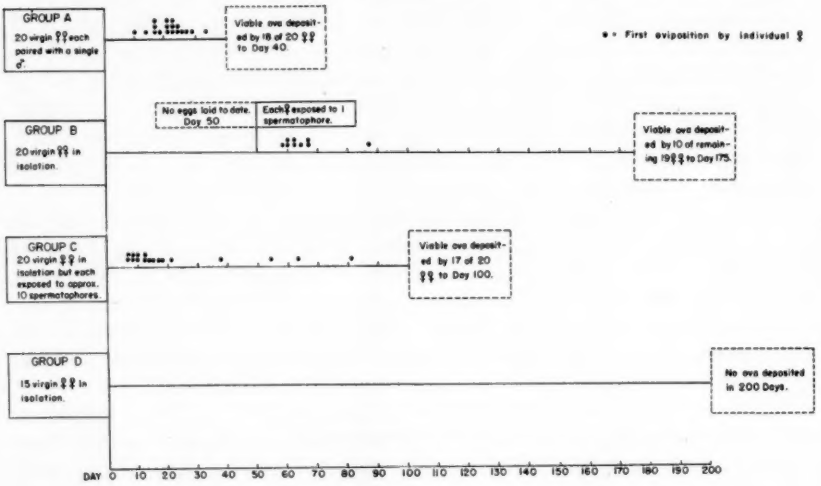
GROUP A
20 virgin ♀♀ each paired with a single ♂.
Viable ova deposited by 18 of 20 ♀♀ to Day 40.
• • First oviposition by individual ♀
No eggs laid to date, Day 50
Each ♀ exposed to 1 spermatophore.
GROUP B
20 virgin ♀♀ in isolation.
Viable ova deposited by 10 of remaining 19 ♀♀ to Day 175.
GROUP C
20 virgin ♀♀ in isolation but each exposed to approx. 10 spermatophores.
Viable ova deposited by 17 of 20 ♀♀ to Day 100.
GROUP D
15 virgin ♀♀ in isolation.
No ova deposited in 200 Days.
DAY 0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200

FIGURE 1. An outline of an experiment on the fertilization and oviposition of the chigger *T.* (*E.*) *splendens;* 4 groups of virgin females, each exposed to a different set of conditions.

males were exposed to approximately 10 spermatophores each at the beginning of the experiment. The first depositions of ova by 13 individuals of this group occurred on days 8 to 21, with the preoviposition periods of 4 females extended to days 38, 55, 63 and 81. *Group D* females were maintained in isolation from males and spermatophores for over 200 days. None of these females laid eggs.

Observations on Spermatophore Deposition

The behavior of male chiggers (*T. splendens*) during the act of spermatophore deposition usually followed a distinct pattern. The male ceased walking, lowered its body until the distended genital plates made contact with the plaster surface. A small ductile mass of material was attached to the substrate, and by elevation of the body, the mass was drawn out into an elongate central filament and usually 2 lateral filaments. At the peak of body elevation, the spherical sperm sac portion was extruded. As the male moved forward, the flexible central stalk assumed a more or less upright position while the more slender lateral filaments became broadly curved. The entire process usually was completed in a few seconds.

In the absence of the female, spermatophores may be deposited in rapid succession, at intervals as brief as 2 or 3 minutes, but the rate of deposition observed was by no means constant. In one instance 12 spermatophores were deposited in a period of just over 2 hours. On the other hand, many males did not produce any spermatophores during periods of observation of 12 consecutive days or more. One

prolific spermatophore-producing male deposited about 520 spermatophores in 34 days.

Other data on spermatophore-production were obtained and recorded while spermatophores were being harvested for the purpose of exposing them to Group C females. Males were regularly isolated for periods of 24 hours in 1-dram tubes; then they were returned to the stender dishes. The production of spermatophores of 70 males (some of which may have been isolated 2 or 3 times) for periods of 24 hours was as follows: 27 did not deposit any spermatophores; 13 each produced a single spermatophore; 29 produced from 2 to 9 spermatophores each; and 1 produced 16 spermatophores. The ages of these males varied from 2 weeks to 3 months.

TABLE I.—*Measurements of Spermatophores*

Species	Range of measurements in microns (4–10 specimens in each category)			
	Length of stalk	Diameter of stalk at midpoint	Length of arms supporting sperm sac	Diameter of sperm sac
Trombicula (L.) pallida	73–95	3–4	34–40	—
T. (L.) n. sp.	104–174	3–5	36–40	—
T. (E.) splendens	192–270	7–12	62–71	30–45
Hannemania sp.	100–125	8–12	46–55	—

Morphology of Spermatophores

The typical spermatophore consists of a main flexible stalk, the base of which is attached to the substrate, and a spherical sperm sac clasped at the apical end of this stalk. Both stalk and sperm sac are translucent and whitish.

Spermatophores of the 4 species studied exhibit certain differences in morphology. The spermatophores of 2 species of *Leptotrombidium* (Figs. 5, 6) are almost indistinguishable except in length of the main stalk as shown by the measurements in Table I. The spermatophores of *T. splendens* (Fig. 1) and *Hannemania* (Fig. 7), representing 2 subfamilies, differ markedly in their structure from each other. *T. (E.) splendens* usually possesses a pair of filaments originating at the base of the main stalk (Figs. 1, 3). Occasionally, an anomalous spermatophore was produced by *T. splendens* although the male that produced the spermatophore illustrated in Figure 4 also produced normal spermatophores. *Hannemania* differs from the others in that the 2 projections which clasp the sperm sac arise from a broad apical expansion of the main stalk.

Insemination by exposure to a single Spermatophore

The behavior of female chiggers before, during, and after the act of insemination usually followed a definite pattern. A virgin female, when encountering a spermatophore, first examined it by tapping lightly and rapidly with the tarsi of the forelegs and sometimes touching it with the gnathosoma. The period of apparent recognition and orientation usually required but a few seconds. If receptive, the female elevated her body and walked over the spermatophore so that the genital opening, with genital plates spread apart, was above the sperm sac. Then seemingly without a pause, she lowered herself onto the spermatophore, sometimes briefly swaying laterally, then departing with the sperm mass. Usually, the sperm sac was completely removed upon proper contact and the sperm thus acquired.

The precise mechanics of the removal of the sperm sac was seen clearly and in minute detail only once. On this occasion, the female was moving toward the observer with the ventral side clearly presented. As the female walked over the stalk bearing the sperm, her genital plates were fully opened, the genital aperture was maneuvered over and onto the sperm sac within a matter of 1 or 2 seconds, the genital plates closed around and completely engulfed the sperm mass which was then pulled free from its stalk. The movements of the female were uninterrupted and exact during her continuous forward motion. From initial recognition to the picking up of the sperm mass, less than 5 seconds had elapsed.

Within a minute or two following the acquisition of sperm, an inseminated female usually stopped walking and a series of expansions and contractions of the body occurred. These movements resembled the body activity which usually accompanies feeding by a nymph or adult, but perhaps they were more vigorous. The violent internal movements were generally succeeded by 3 to 6 jerky, backward movements of the entire body, at intervals of about 1 second. Occasionally, even after the female resumed her normal walking activity, the jerky behavior would again be repeated. On several occasions, following the behavior described, females lowered their bodies onto the substrate and, with the genital plates spread widely apart, appeared to grasp the substrate firmly, thus remaining almost motionless from a few seconds to 5 minutes or more. Breaking of contact with the substrate sometimes occurred with an obvious sudden release suggesting that there had been a firm attachment.

Not all females seemed immediately successful in picking up either all or part of the sperm sacs in their first attempts at insemination. A few succeeded only on the second or third attempt.

Insemination by Exposure to More Than One Spermatophore

In general, observations of females exposed to more than 1 spermatophore, such as in Group C, were fragmentary. In only 1 case was an attempt made to determine the fate of each of 10 spermatophores available to a female at 1 time, and to observe her behavior. In this case, numbers were given to each spermatophore representing the order in which they were picked up or physically altered by the female.

The following is a summary of the fate of these spermatophores: Within seconds after the female was introduced to 10 spermatophores, sperm mass no. 1 was engaged and removed. Sperm mass nos. 2 and 3 were picked up 19 and 20 minutes later and no. 4 was probably acquired between minutes 21 and 23. Following the acquisition of no. 4, the female became progressively more active, appeared "excited" and displayed complete disregard for the remaining spermatophores. At minutes 32 and 35, sperm masses 5 and 6 were brushed off, one each onto the left and right femora of Legs II. Spermatophores 7 and 8 were trampled and their sperm content smeared on the substrate. By minute 57, the female, having again slowed down, properly engaged and picked up sperm mass no. 9. Continuous observations were made for 80 minutes, at which time spermatophore no. 10 remained intact; however, 5 hours later the sperm mass had been removed.

The behavior of the female associated with sperm masses 1, 2, 3 and 4 was typical, including body contractions and expansions, and backward, jerky move-

ments. The behavior associated with sperm masses 5, 6, 7 and 8 was that of hyperactivity and apparent total disregard for the remaining spermatophores. No specific behavior was noticed after sperm mass no. 9 was acquired.

SUMMARY

A method of indirect insemination in chiggers is described. Studies of several species of chiggers demonstrated that adult females are inseminated, not by copulation, but by means of spermatophores. The spermatophore, deposited by the male, consists of a flexible stalk which is attached to the substrate and bears a sac of sperm at the distal end. Insemination occurs when the genital opening, located ventrally on the female, makes effective contact with the spermatophore. The presence of the male is not required for the insemination of the female, nor is the presence of the female necessary for an initial deposition of spermatophores by the male.

An experiment was designed to determine the method of fertilization of *T.* (*E.*) *splendens*. The experiment was initiated with 4 groups of virgin females each exposed to a different treatment or set of conditions. The oviposition of viable eggs occurred in 3 of the 4 female groups. Females in 3 of these groups were exposed to 1 or 10 spermatophores, or paired with males; however, oviposition did not occur in the last group of females which were maintained in isolation for more than 200 days. Non-fertilized females were never observed to lay eggs, and there was no evidence for parthenogenesis.

Numerous direct observations were made on the method, behavior, and rates of deposition of spermatophores by males. Spermatophores of 4 species of chiggers are described. The method of insemination and related behavior pattern of the female are also described.

ACKNOWLEDGMENTS

Our sincere thanks are due to Dr. Phyllis T. Johnson, presently of the U. S. National Museum, for preparation of cytological materials and for her participation in other phases of this study. We are also grateful to Major H. C. Barnett, Dr. M. Hertig, and Dr. H. S. Fuller for their critical reading of the manuscript and also for their interest and suggestions. We thank Mr. Talmadge Neal for his technical assistance, and also members of the Field Unit, Commission on Hemorrhagic Fever, Armed Forces Epidemiological Board, who aided in the collection of the Korean larval chiggers. We wish also to acknowledge Dr. G. W. Wharton who provided us with our original colony of *T.* (*E.*) *splendens*.

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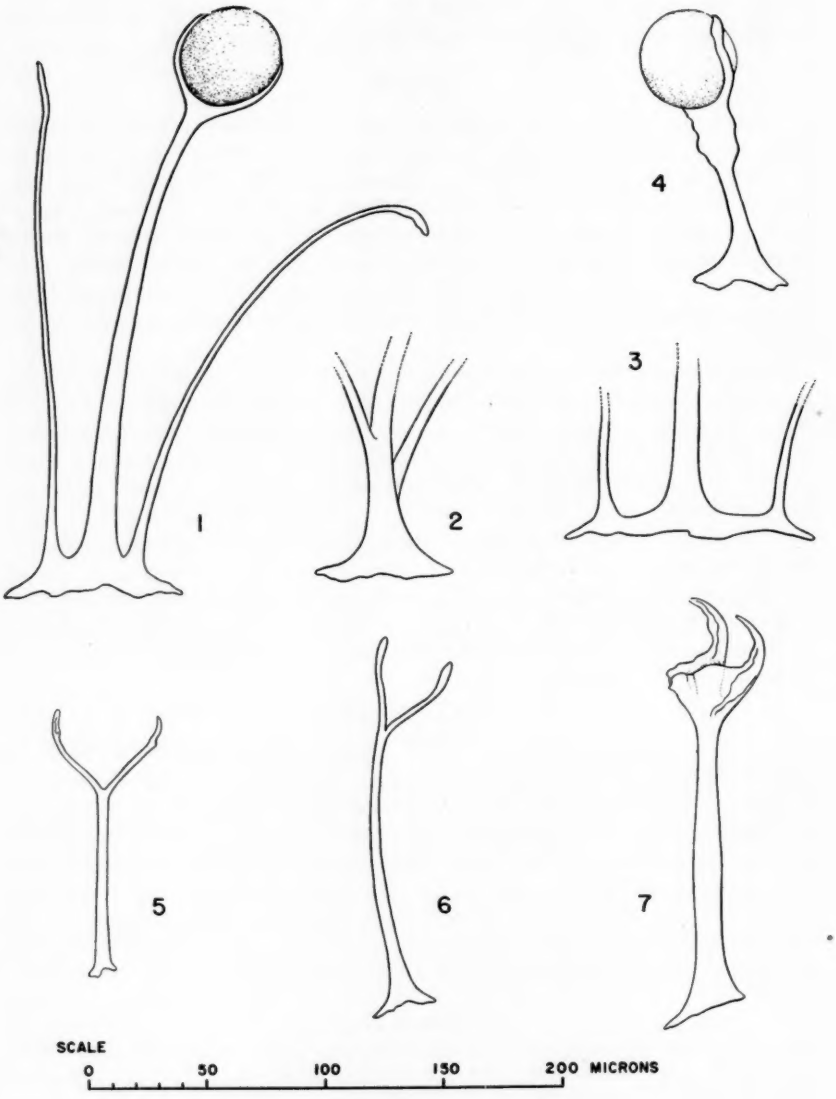
PLATE I



SPERMATOPHORES OF CHIGGERS

FIGURES 1–4. *T.* (*E.*) *splendens,* variations of spermatophores. FIGURE 1. Usual form. FIGURES 2–3. Variations of basal portions. FIGURE 4. Anomalous form.
FIGURE 5. *T.* (*L.*) *pallida* (sperm sac absent).
FIGURE 6. *T.* (*L.*) n. sp. (sperm sac absent).
FIGURE 7. *Hannemania* sp. (sperm sac absent).

BLOOD PYRUVATE LEVELS OF RATS DURING HEMOPROTOZOAN INFECTIONS

ROBERT M. COLEMAN* AND THEODOR VON BRAND

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National Institutes of Health
National Institute of Allergy and Infectious Diseases**
Bethesda 14, Maryland

Data on blood lactate levels of animals infected with trypanosomes are available in the literature (summary in von Brand, 1950), but only one brief reference concerning blood pyruvate levels (Grant and Fulton, 1956) has come to our attention. This is rather surprising since the main metabolic end product of the carbohydrate metabolism of the trypanosomes belonging to the *brucei* group is pyruvic acid (literature in von Brand, 1952).

A more detailed investigation of blood pyruvate levels of trypanosome-infected animals appeared of interest primarily from the standpoint of determining the ability of the host to utilize this metabolic end product of the parasite's metabolism. Utilization by the host had been suggested by Harvey (1949) as a mechanism of salvaging energy originally stored in the glucose molecule and thus preventing parasite autointoxication and host intoxication from excessive pyruvate levels.

In conjunction with these studies on extra-cellular parasites, an investigation of blood pyruvate levels of rats infected with an intra-cellular form, *Plasmodium berghei*, seemed of comparative interest and was included in our studies.

MATERIALS AND METHODS

Trypanosoma equiperdum (NIH strain), *T. gambiense* (Wellcome TS strain), and *T. congolense* (Wellcome FN strain) were maintained in white rats weighing about 200 g, while *T. cruzi* (Brazil strain) was grown in young rats which at the time of the determinations weighed about 45 g. White rats weighing about 100 g were used as hosts for *Plasmodium berghei* (KB G 173 strain). All the rats received a balanced diet (Purina Laboratory Chow and water ad libitum), and the trypanosome-infected rats were not starved prior to the determinations. For comparison, however, several uninfected rats were fasted for 17 hours before being sacrificed. The *P. berghei*-infected rats which were primarily intended for experiments unrelated to the present ones, were starved for 24 hours and either used starving or after having received fructose, meticortelone, or both, as detailed later on.

The control and experimental animals were anaesthetized in etherized chambers. For comparison, a few uninfected rats were anaesthetized by intraperitoneal injections of varying amounts of pentobarbitone but they were not used as controls.

Trypanosome counts were made by means of a hemocytometer immediately before or after collection of the blood sample used for the pyruvate determinations. Glucose was added to the diluent in cases of expected heavy infections to prolong

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survival and thus facilitate counting. The degree of parasitemia was expressed as number of trypanosomes per ml blood. Plasmodia counts were made prior to determinations on Giemsa-stained smears and expressed as percentage of red blood cells parasitized.

Blood samples for the keto acid determinations were obtained from the hearts of several rats infected with *T. cruzi* since the small size of the animals precluded collection of the blood from the aorta, our usual procedure. In all other cases a measured amount of blood, usually 4 ml, was withdrawn from the abdominal aorta by means of a 10-ml syringe containing either 1 ml of heparinized 5% sodium monoiodoacetate in saline or 1 ml of heparinized physiological saline of pH 7.0.

Iodoacetate was used to kill the parasites rapidly and thus prevent as far as possible an increase in pyruvate due to the metabolism of the parasites. This method proved acceptable only in the case of those parasites that do not rapidly produce this acid. With trypanosomes of the *brucei* group, especially in the cases of heavy infections, the time elapsing between the blood entering the syringe and the parasites being killed sufficed to raise the pyruvate level to abnormally high values. For these parasites this procedure was abandoned and the figures resulting from it were discarded. For most experiments a second procedure was chosen. The time for the actual withdrawal of the blood sample into a syringe containing heparinized saline as indicated above varied between 20 and 40 seconds. After withdrawal, the blood was immediately emptied into a test tube preheated to 37° C and the first sample taken and deproteinized. The time elapsing between the blood beginning to enter the syringe and deproteinization of the first sample was accurately timed by means of a stop watch (40 to 90 seconds). The remaining blood was then immediately placed in a water bath of 37° C. At given intervals (1 to 3 minutes, depending upon the number of parasites) further samples, usually 3, occasionally up to 6, were taken. The values obtained were graphed and extrapolated back to zero time. This last value was taken as the true keto acid level of the blood.

Pyruvate was determined essentially according to the direct method of Friedemann and Haugen (1943). It was modified by heating the deproteinized samples for 5 minutes on a boiling waterbath, as suggested by Lichtenbelt and Florijn (1952) and Markees (1955).

RESULTS AND DISCUSSION

The average blood keto acid (pyruvate and α-ketoglutarate) level of uninfected rats (Table I) was somewhat higher than the average usually given, but within the limits found by others (Lu, 1939; Stotz and Bessey, 1942; Goodwin and Williams, 1952). It should be noted that our animals were not starved and that the deproteinized samples had been heated.

It is well known that exercise causes rapid increases in blood pyruvate levels (Johnson and Edwards, 1937; Stotz and Bessey, 1942; Friedemann and Haugen, 1943). Fasting prior to determinations and the use of an anaesthetic which minimizes struggling are important factors in arriving at true resting values. We decided, however, to feed our animals up to the time of the determinations because we were interested in the pyruvate levels as they occur in normally maintained infected animals rather than in the lowest values that could be enforced. Two normal rats were fasted for 17 hours and yielded keto acid levels of 2.3 and 2.6 mg %

respectively, the values lying within the lower range of our control animals receiving food.

Handler (1945) and Goodwin and Williams (1952) suggested the use of a mild barbiturate as an anaesthetic which would largely eliminate undue movements of animals and thus permit determination of true resting values. This procedure causes a very rapid decrease in pyruvate levels (Goodwin and Williams, 1952). Our results with 4 normal starved rats are in general agreement with these findings. Three rats subjected to nembutal anaesthesia for approximately 5 minutes yielded an average value of 4.6 mg % (3.9 to 5.0), while the fourth rat anaesthetized for 20 minutes had a keto acid level of 3.1 mg %. Only this last value was within the range found in our non-fasted etherized rats and we did not feel that nembutal anaesthesia had any advantage over etherization for our purposes.

TABLE I — *Blood pyruvate levels of uninfected white rats and white rats infected with trypanosomes*

	Uninfected	Infected with *Trypanosoma*				
		cruzi	*congolense*	*equiperdum*	*gambiense*	*gambiense**
Number of determinations	14	13	20	25	16	2
Range of parasitemia in millions/ml	—	4-48.5	1-1,162	1.5-1,378	36.5-1,307	1,591; 1,630
Average pyruvate level, mg percent	3.2±0.15†	3.4±0.20	3.4±0.14	3.5±0.17	3.3±0.24	—
Range of pyruvate level, mg percent	2.3-4.1	2.1-4.6	2.4-4.7	2.0-5.3	2.3-5.8	7.0; 12.0

* Terminal parasitemia.

† The figures to the right of the ± sign in this and the following tables are the standard error of the mean.

Investigation of 7 rats showed that heating the deproteinized samples as suggested by Lichtenbelt and Florijn (1952) and Markees (1955) resulted in somewhat higher keto acid levels (3.4 ± 0.36 mg %) than found in unheated samples (2.3 ± 0.25 mg %). Heating did not have any effect on our pyruvate standards. This effect remains to be explained since theoretically one would expect lower keto acid levels from heated than from unheated samples, due to the decomposition of aceto-acetate which may be present in addition to pyruvate and α-ketoglutarate).

Application of our second procedure of determining keto acid levels showed that upon *in vitro* incubation of blood containing trypanosomes of the *brucei* group, a rapid accumulation of keto acids occurred, and that upon plotting the values, straight lines resulted, the slope of which depended on the numbers of parasites present (Figs. 1 and 2). A similar increase was observed in blood samples from *berghei* infections (Fig. 3). The only exceptions occurred in very heavy infections with trypanosomes of the *brucei* group where the curves flattened out after the first 2 or 3 minutes of incubation. This was due unquestionably to the exhaustion of the substrate, glucose, as proven by the pyruvate levels reached and by the fact that raising the initial blood sugar level prevented the flattening of the curves for the periods of observation employed. In extrapolating back to zero time and in calculating the pyruvate production of the trypanosomes, only the straight part of the curves was used.

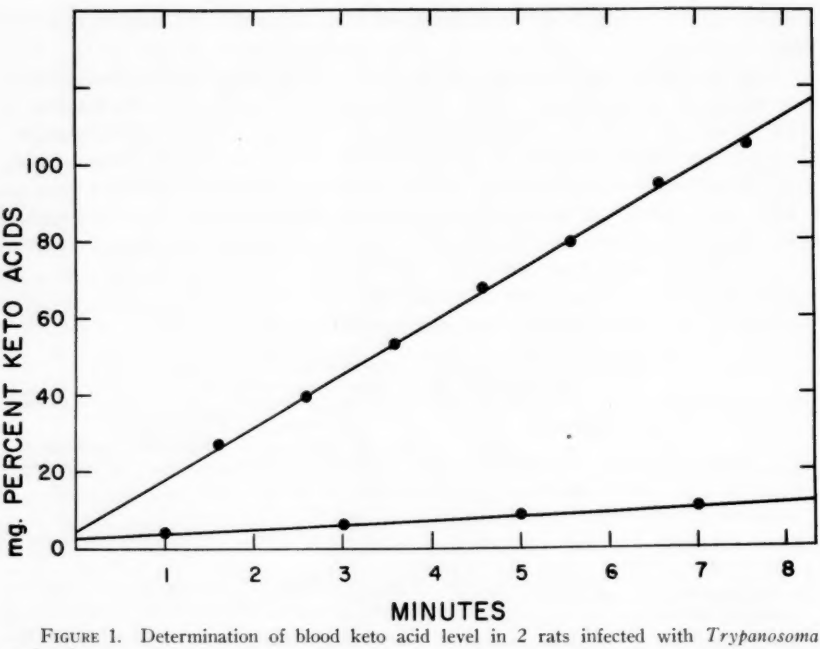

FIGURE 1. Determination of blood keto acid level in 2 rats infected with *Trypanosoma equiperdum*.

Lower line: Parasitemia of 43 million/ml blood. Extrapolated *in vivo* value 3.2 mg %.
Upper line: Parasitemia of 444 million/ml blood. Extrapolated *in vivo* value 5.2 mg %.

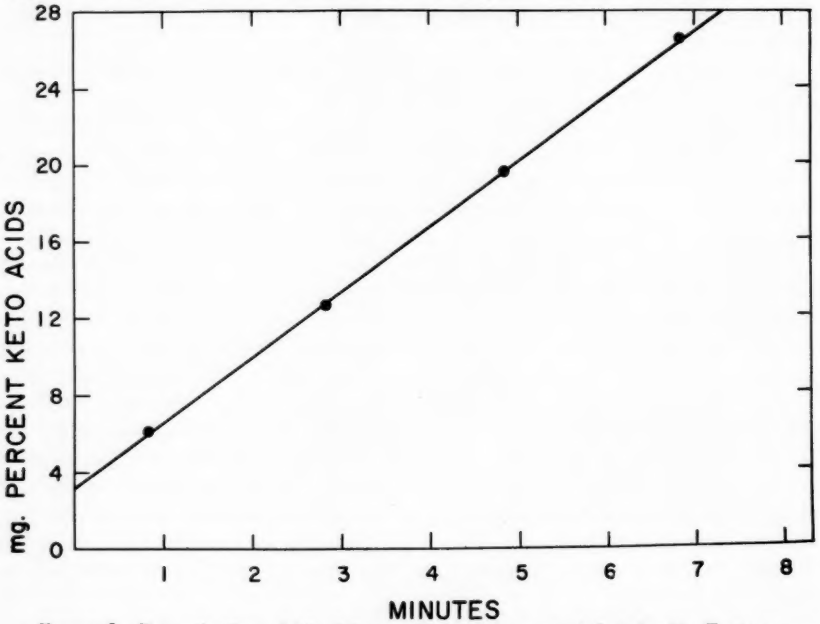

FIGURE 2. Determination of blood keto acid level in a rat infected with *Trypanosoma gambiense*. Parasitemia of 95 million/ml blood. Extrapolated *in vivo* value 3.2 mg %.

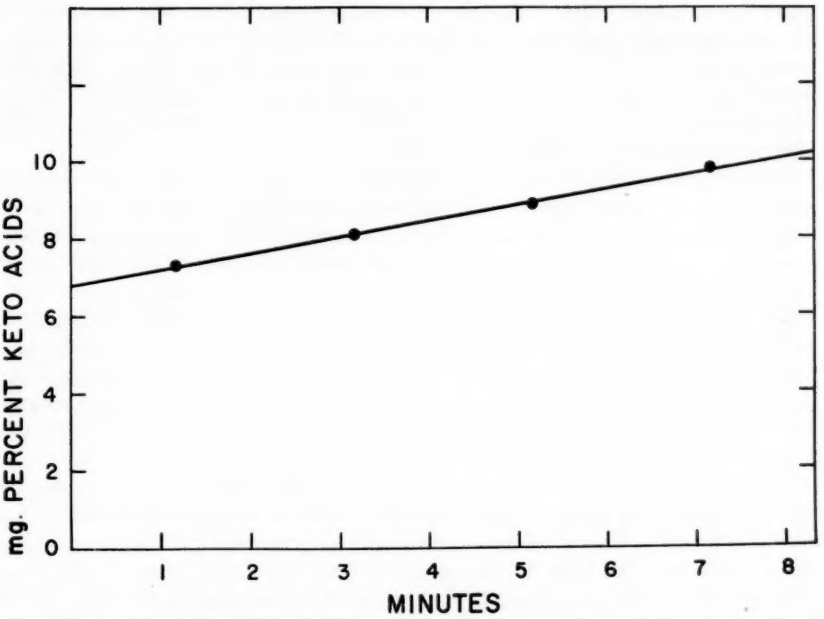

FIGURE 3. Determination of blood keto acid level in a rat infected with *Plasmodium berghei* which had received fructose and meticortelone as detailed in Table III. Parasitemia of 61% of erythrocytes. Extrapolated *in vivo* value 6.8 mg %.

It was possible to calculate the pyruvate production of *T. gambiense* and *T. equiperdum* (Table II). The figures are only slightly higher than expected from the data on sugar consumption and pyruvate production available from previous studies with longer lasting incubation periods (Chen and Geiling, 1945; Agosin and von Brand, 1954 a; von Brand, Weinbach and Tobie, 1955). When using blood containing *T. congolense* or *T. cruzi*, no such calculations were possible because the lines obtained upon plotting were in most cases completely horizontal, indicating that no measurable pyruvate production had occurred during the short periods of incubation. This is not surprising because these forms excrete but very small amounts of the acid (Agosin and von Brand, 1954 b; Ryley, 1955).

TABLE II.—*Rate of keto acid production by trypanosomes expressed as pyruvic acid*

Species	Number of determinations	µg Pyruvate/100 million/1 minute	
		Average	Range
T. gambiense	17	22.4 ± 1.57	12.3 – 36.1
T. equiperdum	25	29.3 ± 1.95	15.6 – 55.5

As mentioned above, in blood taken from *P. berghei* infections a definite but rather slow keto acid production occurred (Fig. 3). Pyruvate accumulation has been observed in incubates of other species of malarial parasites (summary of liter-

ature in von Brand, 1952). However, it must be kept in mind that we incubated parasite-containing whole blood and not isolated parasites. During *berghei* infections, in contrast to trypanosome infections, the nature of the cellular blood elements is greatly altered. It is therefore at present not possible to ascribe deviations from normal blood metabolism to the parasites with any degree of certainty, a point strongly emphasized by Maegraith (1954).

The figures assembled in Table I show that the pyruvate level of the blood remained normal during infection with all species of trypanosomes employed, even when the number of parasites was as high as 1.3 billion/ml. The averages obtained were very similar to the average from control rats, and the extreme high values were, in general, but slightly higher than the highest values observed in the controls. This, then, indicates that the host organism is very efficient in removing the pyruvic acid poured into the blood stream by the parasites, as Harvey (1949) had assumed on theoretical grounds. In one hour, one billion *T. equiperdum* excrete 13.4 mg pyruvic acid. If that many parasites are present in 1 ml blood, the pyruvate level would reach in one hour 1.34% instead of the 0.0035% actually observed if the acid were not removed by the host tissues.‡

It is possible that during the terminal stages of infections with trypanosomes of the *brucei* group the pyruvate level of the blood may be somewhat increased. This seems indicated by the values observed in the two *gambiense* rats listed separately in the last column of Table I. It is probable that Grant and Fulton (1956) were dealing with such terminal stages of *T. rhodesiense* infections when they state "blood pyruvate levels of rats become enormously enhanced during the later stages of an infection." The increases observed by us can hardly be termed "enormous" and we wish to emphasize that we consider our values only as tentative, because with the extrapolation method used by us small errors in timing (5 to 10 seconds) could easily lead to such higher values. On the other hand, in these very high infections the true keto acid level of the blood may be actually slightly higher than indicated by our method. It is evident that during the 20 to 40 seconds required for the blood to enter the syringe, the blood entering the syringe last was longer in contact with the host tissues than the blood entering first and thus probably lost more pyruvate. At any rate, however, it is clear that even in the terminal stages of the infection most of the pyruvate produced by the trypanosomes is removed from the blood by the host's metabolism.

In contrast to the trypanosome infections, a definite increase in average blood keto acid levels was observed in *Plasmodium berghei* infections, but the lowest values were within the normal range (Table III). Because in this infection a very pronounced anemia occurs, the possibility was considered that an unequal distribution of keto acids between blood plasma and formed elements could explain the above observation. In 3 uninfected rats the average keto acid level of the plasma was 4.4 mg % while the whole blood yielded an average of 3.4 mg %. It is evident that this differential in distribution, though possibly a contributing factor, is not pronounced enough to explain the increased average level in *berghei* infections. As shown above, blood infected with *P. berghei* produces some keto acid *in vitro*. This

‡ In this calculation the assumption was made that the flagellates produce identical amounts of pyruvate *in vivo* and *in vitro*. This seems justified in view of the extremely short periods of incubation used. It is hardly probable that immediately upon withdrawal from the host a fundamental shift in metabolism occurs.

production is slow, however, and it appears unlikely that it is directly responsible for the increased *in vivo* levels. It is probable that a metabolic disturbance of the host is the decisive factor. It is well known that increased blood pyruvate levels occur in many diseases, such as many cases of liver dysfunction (e.g. Parida and Kark, 1956, and many others). It may be pertinent to point out in this connection that the liver function of *berghei* rats is markedly disturbed (von Brand and Mercado, 1956). It should also be noted that the various treatments given our rats (fasting, administration of fructose or a steroid) did not have any significant influence on the blood pyruvate levels, an observation which may again be indicative of a pronounced metabolic dysfunction.

TABLE III — *Blood pyruvate levels of white rats infected with* Plasmodium berghei

Number of determinations	Treatment of host	Range of red cells parasitized in percent	Average pyruvate level, mg percent	* S	Range of pyruvate level, mg percent
11	Starved (24 hrs.)	32-71	5.4±0.42	4.9	2.8-7.8
9	Starved (24 hrs.) Fructose (250 mg)	32-75	5.3±0.30	6.2	3.9-6.9
9	Starved (24 hrs.) Meticortelone (3 mg)	32-73	4.8±0.38	3.9	3.4-6.5
9	Starved (24 hrs.) Fructose (250 mg) Meticortelone (3 mg)	33-73	5.4±0.45	4.6	3.0-7.0

Fructose was given orally 3 to 4 hours before the samples were taken.
Meticortelone was given intramuscularly 18 hours before the samples were taken.
For uninfected rats see Table I.

* S = Significance of difference between average pyruvate levels of uninfected and infected rats calculated according to the formula $\frac{M_1 - M_2}{\sqrt{E_1^2 + E_2^2}}$. Any figure larger than 2.0 indicates a significant difference.

SUMMARY

1. The blood keto acid levels of rats infected with pathogenic trypanosomes is essentially normal even in heavy infections (at least up to 1.3 billion/ml) with species producing pyruvic acid at a rapid rate. A somewhat increased level may, however, occur in the terminal stages of these infections.

2. The blood keto acid level of rats infected with *Plasmodium berghei* is definitely increased. This probably is not directly related to the metabolism of the parasites, but rather due to a metabolic disturbance of the host.

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THE LIFE HISTORY OF *CRYPTOCOTYLE CONCAVUM* (CREPLIN, 1825) FISCHOEDER, 1903 (TREMATODA: HETEROPHYIDAE)

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This paper results from a parasitological study of a population of three-spined sticklebacks, *Gasterosteus aculeatus* L., encountered during a hydrobiological survey of the Santa Ynez River directly northwest of Santa Barbara, California. The fish were often so heavily infected with encysted metacercariae (as many as 2,000 cysts in a single fish) that they appeared almost entirely black from the deposition of melanin. Attempts to infect young chicks and ducklings with the metacercaria were successful. The adult trematodes obtained experimentally belong to the family Heterophyidae and the species *Cryptocotyle concavum* (Creplin, 1825) Fischoeder, 1903.

They were assigned to this species since the body is ovoid and the testes are side by side. Thus they differ from *C. jejunum* Nicoll, 1907, *C. echinata* (Linstow, 1879) and *C. lingua* (Creplin, 1825) Fischoeder, 1903, which have linguaform bodies and testes in tandem. Since they possess ovoid eggs they differ from *C. cryptocotyloides* (Issaitchikow, 1923), and *C. quinqueangulare* (Skrjabin, 1923) which have reniform eggs. The vitelline follicles extend more anteriorly than in previously described and figured specimens of *C. concavum*. Considerable variation is found in closely related species as the result of development in different hosts, so this is considered as a variation within the species.

As far as is known, members of the Heterophyidae have similar life histories. The cercariae upon emergence from the snail hosts encyst in fishes, or rarely in amphibians, being transferred passively to the definitive host when the infected fish are eaten. One peculiarity of the heterophyids is the lack of specificity in the final host since, at least experimentally, all members which have been fully investigated develop to maturity in both avian and mammalian hosts. To date the local definitive hosts of *C. concavum* have not been determined although various herons were observed feeding along the stream. According to European workers, the definitive hosts are various aquatic birds. Issaitschikow and Weinberg (1926) identified small heterophyids taken from dogs, cats and rats in the Crimea as *C. concavum*. This would indicate that this fluke is capable of infecting both birds and mammals. The species is fairly widely distributed in Europe but it has not been previously recorded in North America.

SYNONYMY

Cryptocotyle concavum was originally described by Creplin (1825) as *Distoma concavum*. Looss (1899) placed *D. concavum* and the apparently closely related *D. lingua* in the genus *Tocotrema*. Lühe (1899) in the same year erected the genus *Cryptocotyle* but it remained for Fischoeder (1903) to place both *D. concavum* and *D. lingua* in the genus *Cryptocotyle*. Ransom (1920) in his work on the family Heterophyidae retained both in the genus *Cryptocotyle*, but Witenberg

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(1929) in his monographic treatment of the Heterophyidae returned *C. lingua* to the genus *Tocotrema* since he felt that the differences between it and *C. concavum* were of generic significance. Stunkard (1930) and subsequent workers have not recognized these differences as being of generic significance and have suppressed *Tocotrema* Looss, 1899 in favor of *Cryptocotyle* Lühe, 1899. The evidence at hand tends to indicate that *C. concavum* and *C. lingua* are con-generic.

METHODS AND MATERIALS

The observations reported in this paper were made initially on material collected from a small spring-fed stream, Alamo Pintada, near Solvang, California in the spring of 1950. The infected sticklebacks, as previously mentioned, were fed to young chicks and ducklings resulting in numerous sexually mature trematodes within 24 to 48 hours after ingestion of the fish.

The second step was to investigate the snail population of the mile-long stream which was the source of the infected fish. The predominant snail was a small operculate form, *Amnicola longinqua* Gould. Although other snails, *Physa sp. Lymnea sp.* and *Helisoma sp.* were also present, no larval trematodes were obtained from them.

Upon collection and isolation of individual amnicolid snails, it was discovered that these served as primary hosts not only for the pleurolophocercous cercaria of *C. concavum,* but also for the larval stages of an unidentified trematode belonging to the Plagiorchioidea. The latter were small, possessed stylets and short stumpy tails which placed them in the "cellularia group" of Xipidiocercariae of Sewell, 1922. Several hundred *A. longinqua* from the stream were examined during the course of the investigation and approximately 85% were infected with larval trematodes. None of the snails examined, however, was infected with both species of cercariae. Of those infected, approximately 60% yielded stages of *C. concavum* and 40% xiphidiocercariae.

In addition to the infected fish collected in Alamo Pintada, uninfected fish of the same species from other streams were collected and experimentally infected. Moderate to heavy infections resulted, with dark pigment being deposited from the 10th day.

In January 1952, extremely high flood waters completely destroyed the stock of both fish and snails in the source stream. However, in the late spring of 1956 a secondary source of limited infection involving the same snail and fish hosts was found. This source adjacent to the small town of Santa Ynez was a spring-fed stream approximately 2 miles in length. This stream drained into the Santa Ynez as did Alamo Pintada.

The chicks and ducklings used in the experimental infection were obtained from commercial sources. They were all "day old" birds; thus the posssibility of infection from other sources was negligible. Control birds for each experiment were autopsied and found to be negative.

All measurements and figures were taken from living animals. During preservation of the various stages, the attempt was made to fix the material in as natural a condition as possible. Warm Gilson's as well as other standard fixatives were used.

Observations

Larval Stages

Eggs and Miracidia: The eggs are ovoid, somewhat flattened at the opercular end and measure 0.027–0.031 mm in length by 0.016–0.017 mm in width. Measurements of a large number of eggs gave an average length of 0.029 mm and a width of 0.016 mm.

When first laid, each egg contains a cleaving embryo. Eggs were isolated in small stender dishes and periodically examined. The exact time of development of the miracidia was not ascertained. However, at the end of 6 weeks miracidia could be observed moving within the dark brown shell of the egg. Due to the color of the shell, the morphological features of the miracidia could not be observed.

Failure of eggs to hatch in water is characteristic of other heterophyids such as *Metagonimus yokagawai* (Katsurada, 1912) and *Heterophyes heterophyes* (Siebold, 1852) Stiles and Hassall, 1900. Stunkard (1930) also found that in *Cryptocotyle lingua* the eggs evidently had to be ingested before the liberation of the miracidia would occur.

Sporocysts and Rediae: Several hundred snails were examined during periods throughout the year but no sporocyst stages were encountered. Redial stages ranging considerably in size were encountered. The largest ones reached a length of 1.1 mm. The oral sucker and the sac-like gut are prominent and appear to remain functional throughout the entire life span of the redia. The gut remains essentially the same length in the various sizes of rediae and therefore becomes proportionally smaller as the rediae elongate. The shape of the gut varies considerably in the more mature rediae due to crowding and movement of the cercariae.

The rediae (Fig. 1) with an average length of 0.95 mm and width of 0.21 mm are thin-walled elongated sacs usually cream or light yellow-brown in color and lacking locomotory appendages. The gut usually is more heavily pigmented containing dull-brown pigment granules. The number of developing cercariae varies in different rediae but ranges from 10 to 15. The cercariae originate from posterior germinal masses with the more mature cercariae being found at the anterior portion of the redia. These more mature cercariae possess dark, prominent eye spots and a small tail which is devoid of the fin folds found on normally liberated cercariae.

Since cercariae without fin folds on the tail were seen escaping naturally from the anterior birth pore of rediae dissected out of snails and were also found free within the body of the snail, it is probable that further maturation takes place in the tissues of the snail host before they become free-swimming.

Cercariae: Normally liberated cercariae (Fig. 2) measure 0.38–0.51 mm in overall length with the tail comprising slightly over 2/3 of the total length. The width of the body is 0.065–0.078 mm and the tail width 0.03–0.04 mm. Such cercariae possess well developed rectangular-shaped pigmented eye-spots located dorso-lateral to the somewhat rudimentary pharynx. Anteriorly the cuticle is covered with spines which decrease in size towards the posterior part of the body. The anterior or oral sucker is capable of considerable movement. Under a cover glass it and the surrounding area of the body is constantly pulled in and pushed out of the anterior end, exploring in different directions. The ventral sucker is absent as in other cercariae described in this family.

The thick-walled excretory vesicle varies in appearance, sometimes being ovoid, and at other times a thick Y-shaped structure. A mass of germinal cells is present in the median portion of the body just anterior to the excretory vesicle. Between the germinal cells and the eye-spots there is an irregular group of from 16–18 penetration glands. These can best be demonstrated with neutral red vital stain. They are drained by 4 bundles of ducts which can be indistinctly seen passing around the oral sucker terminating along the anterior margin of the dorsal and dorso-lateral lips of the sucker. Cystogenous glands can be seen scattered irregularly along the lateral margin of the body of the cercaria, extending from just caudad of the eye-spots to the region posterior to the excretory vesicle.

The dorsoventral fin fold of the tail begins on the dorsal side a short distance back of the body, extending around the end of the tail ventrally to a point slightly anterior to the middle of the ventral surface. The height of the fin is greatest near the midpoint on the dorsal side and at the terminal portion of the ventral side. At the highest point it is approximately twice the height of the tail in this region. Because of the undulations of the fin, it appears to be stiffened by refractile rods, which are only the crenulations of the fin.

The "ventro-lateral" fin folds of some authors are difficult to interpret. They actually appear to originate somewhat more dorso-laterally from the tail stem and to be recurved ventrally from this source. These fin folds have crenulated edges which appear to be composed of pocket-like portions separated by thin septa one from the other. These pockets are more or less symmetrical on both sides of the straightened tail, usually forming 10 pair, but with considerable variation.

The cercariae swim by a rapid beating of the tail for a period of 2 to 3 seconds followed by a period of quiescence of from 14 to 17 seconds. Factors such as temperature and age after emergence of the cercariae influence the duration of activity and rest periods. When these factors are controlled, the time intervals are remarkably constant. The cercariae are positively phototropic and swim up to the surface of the water and then settle slowly to the bottom of the container. This method of swimming is common to all pleurolophocercous cercariae which have been described.

Rothschild (1938) found it difficult to distinguish between related pleurolophocercous cercariae. She used such characters as the proportional extent and nature of the caudal fin fold, pigmentation of the body and the behavior of the larvae. She stressed that such characteristics as the length of free swimming life, the relative duration of passive and active periods of swimming, the intermediate host used and the site of encystment in these hosts, as well as other patterns of behavior, were all important in distinguishing between the cercariae of this group.

Encystment of the cercariae: When excised portions of the fin or tail of uninfected *G. aculeatus* were placed in watch glasses containing free-swimming cercariae, the penetration and early cyst formation was easily observable. The cercariae, upon coming in contact with the excised fish structures, continue to beat their tails constantly for several minutes at a time. This beating tends to keep the anterior sucker in contact with the surface of the fish until penetration is initiated. The backwardly directed spines, especially large on the anterior end, and the protrusible nature of the sucker and surrounding cephalic area both aid in penetration. It is probable, on the basis of what is known concerning the penetration of other

cercariae that the contents of the penetration glands aid in penetration but such activity was not observed. The larvae which penetrate the fins and tail tend to encyst along the fin rays. However, encystment is not confined to these areas. Once a larvae reaches a satisfactory location, it begins a rotatory movement, continuing this activity for from 2 to 3 hours as the cyst wall becomes increasingly thicker. The entire process of encystment requires from 2½ to nearly 4 hours, after which time the larva becomes semi-quiescent.

In addition to encystment experiments with excised fins and tails from *G. aculeatus,* experimental infections with living fish were attempted. *Gambusia affinis* and *Cyprinus carpio,* which also occurred naturally in the source stream, and uninfected sticklebacks from other sources were placed in an aquarium with numerous snails shedding cercariae of *C. concavum.* Moderately heavy to heavy infections were obtained in 2 to 3 weeks in *G. aculeatus,* but not in either of the other 2 species of fish. This indicated an intermediate host specificity at least in regard to the fish which occurred naturally in this source stream.

The *G. aculeatus* infected in this experiment developed the typical dark mottling caused by masses of melanophores. The melanin pigment was deposited by the fish in the form of a capsule over encysted metacercariae. By the end of 4 weeks, when the fish were fed to experimental ducklings, the color was almost as dark as in natural infections. In extremely heavy natural infections, the cysts with their surrounding melanophores were so abundant that the fish were almost entirely black. This pigmentation sometimes extended into the cornea so that often a fish was partially blind in one or both eyes.

Metacercariae: (Fig. 3) Measurements of 25 newly formed cysts average 0.095 mm by 0.081 mm. In these, the larva is curled inside the cyst wall. However, after 2 weeks when the cysts average 0.29 mm by 0.21 mm, the elongated larvae are folded upon themselves often in the form of a U. The cyst wall is unstainable with vital stains and is extremely tough, seemingly being composed of concentric layers. The larvae are active within the cysts, moving within the limited confines of the cyst wall. The anterior sucker and the bulb-like pharynx are clearly discernible in the encysted larva. The irregularly shaped excretory vesicle is often in view. Rudiments of the reproductive system can be seen anteriad to the vesicle.

Standard preparations of digestive enzymes were used in attempts to digest away the cyst wall but were never successful. Careful mechanical encystment, aided by pressure exerted by a cover slip, furnished most of the material for studies of the metacercariae. Before measurements were taken or camera lucida sketches made, it was ascertained that the liberated metacercaria were active and uninjured.

The primordia of all the internal organs are present in the excysted metacercariae. These structures are in the same relative position as in the adult worm, differing only in size and maturity. It is not possible to see distinct vitellaria at this stage.

From preliminary examination of the flame cell pattern, it is evident that the anterior groups of flame cells of *C. concavum* metacercariae agree with those reported by Stunkard (1929) for the same stage of *C. lingua.* Stunkard found the formula of $2 \times [(3+7+7)+(7+7+7)]$ for the metacercarial stage of *C. lingua.* In *C. concavum* it is extremely difficult to determine the exact relation of the last 3 groups of flame cells. The crowded posterior half of the body and the presence

of at least 1 ciliated patch within each of the 2 primary collecting tubules leading into the excretory vesicle makes interpretation of the distribution of the excretory system in this region difficult. It seems probable, however, that *C. concavum* will agree with *C. lingua* on the basis of the preliminary work.

Adult

Sexually mature worms were obtained within 24 to 48 hours after infection of ducklings and chicks. These worms contained fewer ova and were slightly smaller than worms allowed to develop for 8 to 11 days. The worms were found in the anterior portion of the intestine in 14-day infections. Infections of 18 days yielded fewer adult worms and those recovered were usually more abundant in the posterior portion of the intestine. This seemed to indicate that the experimental hosts were perhaps eliminating the infection. The feces were not checked either for eggs or worms. Only the older worms were used for drawing and measurements.

The intestine of infected experimental hosts possessed a characteristic mottled appearance with reddened areas. In regions where adult trematodes were abundant, the inner intestinal wall was denude of villi.

In these studies, several hundred adult specimens were examined and it was noted that while as a rule *C. concavum* is shorter and broader and in general smaller than *C. lingua,* nevertheless many living worms approached the latter in shape. All living adults were longer and narrower than preserved specimens.

The body of the adult (Fig. 4) while quite mutable in shape, is usually ovoid, 0.6–0.95 mm in length and 0.5–0.8 mm in width. The oral sucker measures 0.09–0.10 mm in diameter. There is a short prepharynx followed by a rounded pharynx 0.060–0.064 mm in diameter. The bifurcation of the intestine occurs about midway between oral sucker and acetabulum. The intestinal ceca curve gradually toward the sides of the body, not approaching the edges until opposite the testes. At this point they follow the curvature of the body toward the mid-line but never come in contact with the excretory vesicle.

The slightly lobed testes, somewhat transversely elongated, are side by side, near the mid-line of the body just anterior to the ends of the ceca. Occasionally a portion of one or both testes extend across the mid-line but they never appear tandem.

The rounded seminal receptacle is situated anterior and somewhat to the left of the right testis. The ovary, irregularly ovoid or slightly lobed, is anterior to the right testis and lateral to the seminal vesicle. [As Witenberg (1929) points out in a foot note on page 181, "Several authors describe the ovary as situated in other places but their ascertations are probably erroneous."] The uterus, composed of few loops, is confined to the intercecal space between the genital complex and the acetabulum. The number of eggs ranges from 6 in young worms to 68 in worms 18 days old. The vitelline follicles are extracecal extending anteriorly to the level of the bifurcation of the gut. Follicles occupy the intercecal space anterior to the acetabulum. The fine vitelline ducts converge into tranverse ducts at the level of the space just anterior to the testes. The acetabulum is situated in the anterior wall of the spacious, muscular genital sinus, located near the middle of the body. The genital ducts open into the center of the genital sinus. The well developed seminal vesicle in the form of an elongated S is situated dorsal to the uterine coils and posterior to the genital sucker.

DISCUSSION

The discovery of the cercaria of *Cryptocotyle concavum* emphasizes the difficulty of differentiating between related species of cercariae on the basis of morphological characters alone. In the case of the pleurolophocercous cercariae, as in other groups of cercariae, it is frequently almost impossible to detect constant morphological differences between closely related species. Cable and Wheeler (1939) pointed out that it is impossible to identify positively many of the described species of pleurolophocercous cercariae on the basis of existing descriptions, emphasizing that "in all cases, the living cercariae must be studied." Marked differences in the behavior patterns, reaction to various stimuli, snail hosts and organisms in which they encyst are often more reliable in differentiating species than the purely morphological approach.

Cercaria lophocerca Filippi 1857, redescribed by Dubois, 1929 from *Bithynia tentaculata* in Europe and *Cercaria Indicae* VIII Sewell, 1922 from *Melanoides tuberculatus, Acrostoma variable, var. spiniferum* and *Digonostoma cerameopama* in India are morphologically very similar to the cercaria of *C. concavum*. However, until their life cycles are known they can not be considered identical.

There are slight morphological differences such as a slightly smaller size and a characteristic difference in the distribution of the fin fold on the tail which separates *Cercaria Indicae VIII* Sewell, *Cercaria lophocerca* Filippi and the cercaria of *C. concavum* from the marine cercaria of *C. lingua* described by Stunkard (1930).

Issaitschikoff and Weinberg (1926) found infected fish, *Gobius ratan* Nordman (3 infections in 2,730 fish examined) in which metacercariae identified by them as *Cryptocotyle concavum* were encysted in the gills. They also reported an unencysted, mobile but obviously immature adult in the intestine of the fish *Trachurus trachurus* L. If the latter identification were correct this undoubtedly would represent development in an abnormal host, since they stated that the previously reported hosts were various aquatic birds, dogs, cats, and the Norwegian rat in the Crimea. Nicoll and Small (1909) found metacercariae which they regarded as the larval form of *C. concavum* encysted in young plaice, *Plueronectes platessa*. The descriptions of the excysted metacercariae and immature adults from fish are morphologically similar to the same stages described in this paper differing only somewhat in size. This difference may not be significant since these larvae are extremely mutable.

Thus we have the anomaly of cercariae from fresh water snails, and metacercariae in at least 2 instances from marine or brackish water fishes, both being identified as larval stages of *C. concavum*. This raises interesting conjectures concerning the ecological distribution of members of the family Heterophyidae. On the basis of described life histories of various representatives of the family it is known that some species utilize fresh water, some brackish water and others marine snails as the molluscan host. The various adults have similarly been reported from either fresh water or marine hosts. The significant question is thus raised: Is it possible for a given species to utilize both fresh water and marine snails as the molluscan host? The American species of *Cryptocotyle* would furnish ideal material for investigations leading to answers to this puzzling and fundamental question.

Stunkard and Shaw (1931) investigated some phases of the problem of the origin and distribution of closely related trematodes that occur in marine and fresh

water hosts. They felt that migration of hosts and transfer to new hosts were the only explanations that appeared tenable. They also felt that the essential factor was the ability of the free-swimming cercariae to live and remain infective in new environments.

It would appear equally feasible to attack the problem from the standpoint of the eggs, especially in a family where the eggs must of necessity be ingested by the snail host prior to hatching.

SUMMARY

Certain important stages of the life history of *Cryptocotyle concavum* are described. The snail host is *Amnicola longinqua* Gould, an operculate, fresh-water form. The cercariae develop in rediae and no mother rediae or sporocyst stages were found. A description of the pleurolophocercous cercaria pointed out the difficulty of distinguishing between closely related species within this group on purely morphological grounds. Natural infections of *Gasterosteus aculeatus* L., the three-spined stickleback, as well as experimental infections of this small fish were utilized in infecting young chicks and ducklings to obtain numerous adults of *C. concavum*. The metacercariae were described and preliminary work was carried out on the excretory system of excysted forms. The problem of the origin and ecological distribution of members of the family Heterophyidae was discussed. Specific proposals for investigation of this problem were made.

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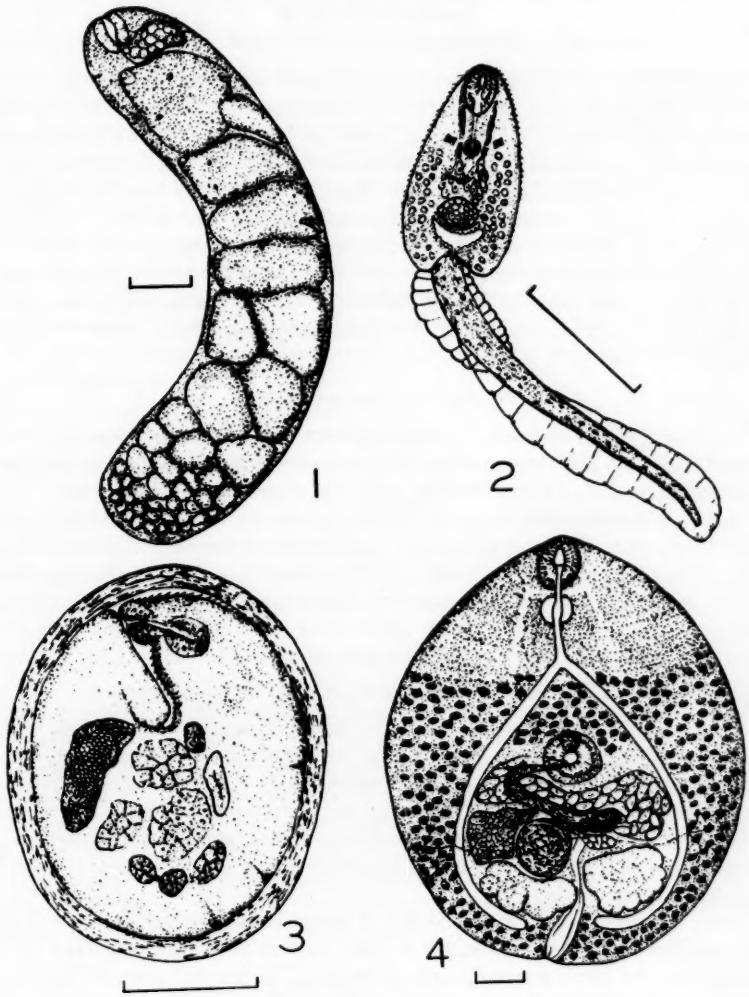
PLATE I



EXPLANATION OF PLATE

Each scale represents 0.10 mm.
FIGURE 1. Redia.
FIGURE 2. Cercaria (Dorsal view).
FIGURE 3. Metacercaria in cyst.
FIGURE 4. Adult (Ventral view).

THE EFFECT OF FURAZOLIDONE* ON PIGEON TRICHOMONIASIS DUE TO *TRICHOMONAS GALLINAE*

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The nitrofuran, furazolidone, when administered at appropriate levels, has been shown to enhance the growth of chickens by Harwood and Stunz (1954), by Berg, Hamilton, and Bearse (1955), and by Libby and Schaible (1955). Francis and Shaffner (1955) reported it to have no adverse effects on egg-production, hatchability, or shell-quality. Harwood and Stunz (1954) found that it reduced the mortality in poults infected with *Histomonas meleagridis* and in chicks with *Eimeria tenella.* Mangrum, *et al* (1955) found it to be similarly beneficial in poults infected with *Hexamita meleagridis.* Lucas (1955) reported a reduction in mortality and morbidity in an epizoötic among chickens caused by *Salmonella gallinarum.*

The above beneficial results indicated that furazolidone should be tried in pigeon trichomoniasis due to *T. gallinae.*

MATERIALS AND METHODS

The drug was administered in gelatin capsules which were pushed gently into the pigeons' crops. A full course of the drug consisted of the particular amount under experimentation (i.e., 200 mg, 100 mg, etc.) administered daily for 7 days.

The trichomonads were those in chronic carriers, as well as the highly virulent Jones' Barn strain, the latter given experimentally to the writer's *Trichomonas*-free birds. The pigeons were 1) carriers of *T. gallinae* from the Frank Foy Farm, Clinton, Iowa, and 2) *Trichomonas*-free homers from the writer's loft (Stabler, 1954).

OBSERVATIONS

Experiments involving naturally infected carriers of T. gallinae.

Dosages of 200, 100, and 50 mg of the drug per day were first tried, using 2 pigeons for each dose. These levels proved highly toxic, all birds showing convulsions and much weight loss. Of the 6 pigeons, only 1 (at 50 mg) survived; the others died in convulsions. The survivor, having received 350 mg in all, appeared grossly normal on the 36th post-treatment day (ptd), and remained negative for trichomonads. Of the remaining 5, 4 became negative for *T. gallinae;* 1 was not checked.

Next, 10 carriers were given 35 mg of furazolidone per day each for 7 days. This dose proved still too toxic, for 5 birds died in convulsions between the 1st and 3rd ptd. Of the 5 survivors, only 1 remained normal throughout; the other 4 showed varying degrees of toxicity. On the 25th ptd, 4 of the survivors were negative for trichomonads; 1 still had *T. gallinae.*

Two pigeons were then each given 25 mg of furazolidone per day for 7 days. These became only mildly ill and easily survived the total dosage of 175 mg each. They were negative for *T. gallinae* on the 19th ptd.

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* Furazolidone is the generic name of Furoxone® (N-(5 nitro-2-furfurylidene)-3-amino-2-oxazolidone) and is made by Eaton Laboratories, Norwich, New York.

Twelve pigeons next received 10 mg of the drug per day each for 7 days. All survived the 70 mg total with no apparent toxic effect. One small bird was *Trichomonas*-positive when killed by its cage mates on the 3rd ptd. Of the other 11, 2 were negative and 9 were still positive for *T. gallinae* on the 13th ptd.

Experiments using the virulent Jones' Barn T. gallinae *in clean pigeons.*

This strain of *T. gallinae* has a history of having killed nearly all non-immune pigeons into which it has been put (Stabler, 1954). Fifteen such *Trichomonas*-free birds were given orally approximately 10,000 Jones' Barn trichomonads each. Three days after inoculation, 9 of these received the 1st of a 7-day series of 10 mg of drug per day. Six served as untreated controls. The controls all died, at an average of 5.5 days post-infection.

Of the 9 treated pigeons, 2 died of severe *T. gallinae* trichomoniasis. One died in typical toxic convulsions on the 2nd ptd, but showed no pathology due to *T. gallinae.* Six birds survived, and showed neither drug toxicity nor trichomonad-induced pathology by the 13th ptd. Only 1 of the 9 treated birds became negative for *T. gallinae,* although 6 were presumably saved from a *Trichomonas*-caused death by the furazolidone.

As a check on the virulence of the trichomonads in these positive survivors, an inoculum was made from the oral contents of 4 of them and given to 2 clean birds. They died on the 4th and 6th days after inoculation, respectively.

SUMMARY

Furazolidone was capable of removing *Trichomonas gallinae* from naturally infected pigeons. Effective therapeutic levels, however, were too generally accompanied by considerable weight loss and convulsions, indicating severe toxic effects, frequently ending in death. Dosages which were tolerated with mild or no symptoms were usually ineffective in removing *T. gallinae* from chronic carriers.

When a highly virulent strain of *T. gallinae* was given to 9 non-immune pigeons, furazolidone at a generally tolerated level was found capable of preventing death due to trichomoniasis in 7 of them. One of the 7 subsequently died of drug toxicity. The drug was unsatisfactory for radical cure of infection with *T. gallinae,* however, as only 1 of the 9 birds became negative. Furthermore, the trichomonads from 4 of the surviving positive pigeons were given to 2 clean birds. They died at 4 and 6 days, respectively.

One positive survivor was mated to a positive cock and she has successfully produced several sets of squabs. This indicates that furazolidone did not adversely affect fertility, egg production, or hatchability.

ACKNOWLEDGMENT

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RESEARCH NOTE

THE EARTHWORM, *EISENIA LONNBERGI*, A NEW INTERMEDIATE HOST FOR SWINE LUNGWORMS

The role played by earthworms in the transmission of the lungworms of swine was first demonstrated by Hobmaier and Hobmaier (1929, Tierarztl. Wchnschr. **80**: 365; 433) and was confirmed by Schwartz and Alicata (1929, J. Parasitol. **16**: 105). According to Alicata (1935, U. S. Dept. Agric. Tech. Bull. **489**: 96 pp.) 5 species of earthworms have been determined experimentally to act as intermediate hosts of the parasites, namely, *Eisenia foetida, Allolobophora caliginosa, Lumbricus terrestris, L. rubellus,* and *Bimastus tenuis.* The purpose of this note is to record the experimental infection of another species of earthworm with swine lungworms.

The earthworms in question were collected at water level from a swamp having a heavy clay bottom covered with well-decayed leaf litter, which was located in the vicinity of Beltsville, Maryland. The specimens were approximately 10 cm long and were brownish violet in color. They were identified as *Eisenia lonnbergi* after comparison with specimens belonging to this species collected from a swamp near Mount Vernon, Virginia, which had been deposited in the U. S. National Museum (Cat. No. 19-314). This earthworm has also been collected from Savannah, Georgia, Raleigh, North Carolina, Petersham, Massachusetts, Chapel Hill, North Carolina, Woodbridge and Cockaponsett, Connecticut, and from Tennessee.

Specimens of *E. lonnbergi* were exposed to a mixture of embryonated eggs of the two common species of lungworms of swine, *Metastrongylus elongatus* and *Choerostrongylus pudendotectus.* About 3 weeks later infective larvae of these lungworms were recovered from the exposed earthworms, whereas unexposed earthworms remained free of infection. These results demonstrated that the larvae of one or both of these lungworms can develop to the infective stage in this earthworm. Since *E. lonnbergi* is found predominantly in a swampy habitat, it may be an important factor in the establishment of heavy swine lungworm infections in the Eastern United States, particularly in areas where hogs have access to swampland.—DONALD A. DAYTON, *Animal Disease and Parasite Research Branch, Agricultural Research Service, Beltsville, Maryland. Present address: East Glacier Park, Montana.*

THE EFFECT OF AZASERINE AGAINST *PLASMODIUM LOPHURAE* IN CHICKS AND ATTEMPTS TO ANTAGONIZE THIS EFFECT WITH METABOLITES*

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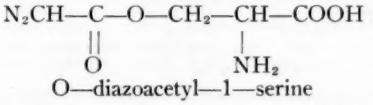
Azaserine is an antibiotic of the following structure (Fusari *et al,* 1954).



O—diazoacetyl—l—serine

It is an amino acid analog, has antitumor activity (Sugiura and Stock, 1955), acts as an antimetabolite with certain microorganisms *in vitro* (Kaplan and Stock, 1954), and has suppressive activity in avian malaria (Coffey *et al,* 1954). These facts encouraged further study of the antimalarial activity of azaserine and suggested that the testing of various metabolites for ability to antagonize its activity *in vivo* might yield useful information on the physiology of the plasmodia and biological activity of the drug. The results of such work are reported here.

MATERIALS AND METHODS

The 12A Strain of *Plasmodium lophurae* in white Leghorn chicks served as the test infection. Infections were induced by the intravenous injection of parasitized blood taken from donors with acute infections. The chicks were 6 to 8 days old when infected and were given an inoculum containing 1×10^9 parasites/kg of body weight. Except as otherwise noted, the test substances were administered in a diet of commercial mash for 5 days, starting the day before the birds were infected with parasites and continuing until all blood smears were made. The effects of therapy were estimated from the apparent degree of suppression of the parasitemia, which was computed from data on groups of treated and untreated chicks studied in parallel. Quinine hydrochloride served as the reference drug and all dosages of it are expressed as the free base. Detailed descriptions of these procedures have been presented elsewhere (Thompson *et al,* 1953). The examination of substances for ability to interfere with the antimalarial activity of azaserine was conducted as in previous antagonism studies (Thompson *et al,* 1953a). Briefly, this involved the testing of known ratios of the potential antagonist and azaserine in combination along with the necessary controls: untreated, azaserine alone, and the potential antagonist alone. Three to 6 birds were employed in each test group.

RESULTS

Antimalarial Activity of Azaserine.

Data comparing the effects of azaserine and quinine in drug-diet treatment are shown in Table I. Both drugs suppressed the parasitemia in each of 10 experi-

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* The authors are indebted to Dr. Alexander M. Moore for assistance.
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ments. The mean degree of suppression at equivalent dose levels of each drug varied considerably, owing mainly to the use of only 3 chicks per treatment group. The severity of the infections varied considerably: the mean percentage of infected erythrocytes ranged from 18.8 to 47.7. This variation resulted primarily from differences in the immediate passage history of the parasite strain; higher infection levels developed when it was passed rapidly many times before use than when it

TABLE I — *Effects of azaserine and quinine in drug-diet tests. Treatment was started one day before infection and continued until blood smears were made on the 4th day after infection*

Exp. No.	Untreated* Mean % CP†	Azaserine* Mean Intake Mg./Kg. Day	Azaserine* Mean % CP	Quinine* Mean Intake Mg./Kg. Day	Quinine* Mean % CP
298	39.9	45●	13.8	68••	9.5
				96††	0.4
303	18.8	58••	6.0	62••	8.0
				100††	4.3
305	22.0	31§	5.6	62••	16.8
		56••	5.0	106††	1.5
307	23.6	31§	5.8	63••	5.6
		63••	5.3	108††	3.5
309	31.2	15‡	25.5	35▲	23.8
		49••	9.2	60••	14.7
				102††	7.5
310	24.1	28§	11.6	42▲	22.4
		51••	8.3	58••	12.1
				100††	0.7
311	31.9	23§	12.9	50••	13.5
				94††	0.8
312	41.9	19♦	29.7	37▲	35.1
				54••	25.6
				93††	9.3
313	47.7	15‡	25.6	61••	25.9
		21♦	24.8	112††	3.4
		32§	25.7		
315	27.9	46••	6.3	64••	20.0
		46••	9.0	99††	3.4
316	33.4	26§	15.5	60••	8.9
		26§	21.9	101††	5.5
		50••	10.5		
318	33.2	24§	20.6	58••	20.2
				88††	8.5

* Three chicks per treated group and 5 to 7 (usually 6) per control group.

† Percentage of erythrocytes parasitized.

Percentage of drug in diet – ‡ = 0.0075; § = 0.015; ♦ = 0.01; ▲ = 0.02; ● = 0.025; •• = 0.03; †† = 0.05.

was passed rapidly only once or twice before use. Despite these variations, plotting on probit paper of the parasitemia suppression against drug dose showed a linear dose-response relation for both azaserine and quinine (Fig. 1). The dose-response regressions, shown in the figure, were computed by the least squares method. Examinations of these regressions (Finney, 1952) showed that the slope of azaserine was significantly less ($P = 0.05$ to 0.02) than the slope of quinine. This difference prevented the assignment of an overall potency ratio. Hence valid comparisons could be made only at specified levels of effect. At the ED_{50}, azaserine

was 2.4 times as active as quinine; the respective ED_{50}'s of 25 and 59 mg/kg/day were significantly different ($P = < 0.001$). However, at the ED_{75} azaserine was only 1.3 times as active. Although comparisons at higher levels of effect were not warranted from these data, earlier work by the authors (Coffey *et al,* 1954) had indicated that at the ED_{90} roughly equal amounts of the drugs are required.

The comparative effects of azaserine and quinine when they were given intraperitoneally are shown in Table II. The drugs were given twice daily (approximately 3 hours before and after blood smears were made) on the 1st through the 4th day after infection. The testing was done in 6 experiments, which typically

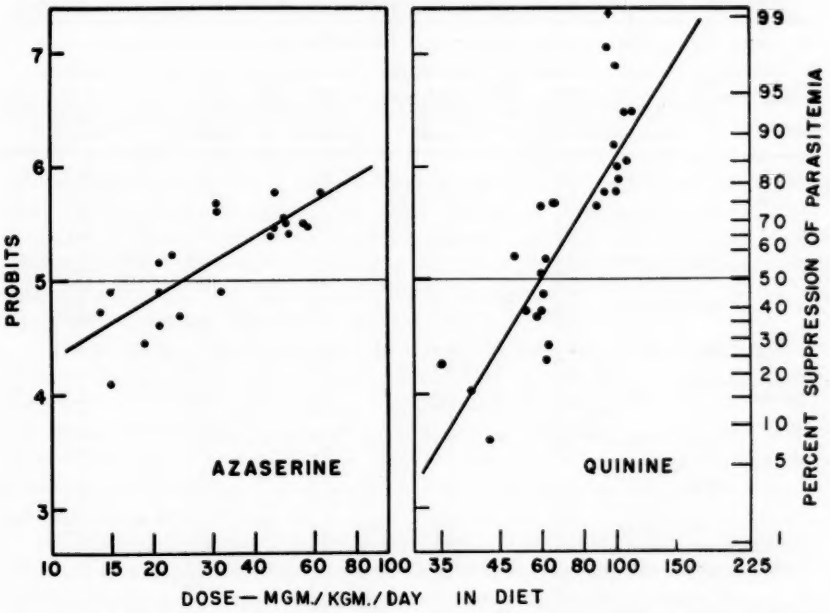

FIGURE 1. Comparison of the parasitemia suppression by azaserine and quinine in drug-diet tests.

included 6 untreated controls and groups of 3 chicks on 1 or more dose levels of the drugs. Space considerations and the sources of variability mentioned in the drug-diet tests encouraged the compilation of the various replicates, as shown in the table, for the most effective comparison of the drugs. Although no attempt was made to determine the statistical significance of these data, several conclusions either are obvious or are reasonably supported. The data show clearly that azaserine has suppressive activity. By inspection of probit plots (not shown) of the mean degree of suppression on the 4th and 5th days relative to dose level, this activity can be compared roughly with that of quinine. The estimated ED_{50}, ED_{75} and ED_{90} in mg/kg/day were for azaserine 8, 15 and 22, and for quinine 7, 10 and 14. Thus, as in the drug-diet tests, the apparent dose-response curve was flatter with azaserine than with quinine; this again prevented determining an overall potency ratio. The tabulated data also indicated that azaserine and quinine acted

at about the same rate when they had a similar degree of suppressive effect. This was most clearly apparent with respective doses of 7.5 mg/kg/day. Neither drug acted rapidly enough for consistent effect within 3 hours after the first dose (see percent C.P. on 1st day after infection). However, suppression was apparent, particularly at the higher dose levels, following the 3rd dose (2nd day) and was progressively greater after the succeeding doses. Finally, it should be noted that azaserine was more toxic for chicks than quinine: repeated azaserine doses of 60 mg/kg/day were lethal.

TABLE II — *Effects of azaserine and quinine when given intraperitoneally*. The drugs were given twice daily (approximately 3 hours before and after blood smears were made) on the 1st through the 4th day after infection.*

Drug	Dose Mg./Kg. Day	No. Birds Treated / Controls	Days After Infection: 1 — % CP† Treated / Controls	1 — % Supp.	2 — % CP Treated / Controls	2 — % Supp.	3 — % CP Treated / Controls	3 — % Supp.	4 — % CP Treated / Controls	4 — % Supp.	5 — % CP Treated / Controls	5 — % Supp.
Azaserine	3.5	6/13	0.7 / 0.9	19	2.3 / 3.2	28	6.4 / 7.7	17	15.4 / 15.6	1	19.5 / 28.2	31
"	7.5	9/19	1.1 / 1.2	8	2.5 / 4.2	40	6.3 / 8.5	26	13.1 / 22.7	42	16.0 / 35.6	55
"	15.0	9/18	0.9 / 0.8	0	1.9 / 3.0	37	4.4 / 8.3	47	6.8 / 23.4	71	7.6 / 30.6	75
"	30.0	15/25	0.6 / 0.9	29	1.3 / 2.9	55	1.8 / 7.7	77	1.5 / 19.1	92	0.8 / 28.0	97
"	60.0	6/6	0.7 / 0.6	0	0.5 / 1.7	74	0.6 / 5.4	90	‡		‡	
Quinine	7.5	6/12	1.3 / 1.3	0	4.0 / 5.5	27	9.4 / 13.7	31	15.6 / 33.6	54	20.9 / 45.9	54
"	8.5	5/6	3.0 / 2.3	0	6.0 / 7.0	18	12.7 / 20.1	37	15.5 / 47.7	68	19.3 / 57.6	66
"	10.0	4/6	2.1 / 2.3	9	3.2 / 7.3	56	8.2 / 20.1	59	13.3 / 47.7	72	16.5 / 57.6	71
"	15.0	15/24	0.8 / 0.9	16	1.0 / 3.1	68	1.4 / 8.3	83	1.1 / 22.2	95	0.6 / 31.1	98
"	30.0	15/25	1.1 / 1.0	0	1.0 / 2.7	63	0.5 / 7.5	93	0.3 / 18.0	98	0.1 / 27.0	>99
"	60.0	9/13	0.7 / 0.8	18	0.7 / 2.1	66	0.4 / 5.8	94	0.2 / 14.0	99	0.1 / 21.5	>99

* These data were compiled from 6 experiments. Typically each experiment included 6 untreated controls and groups of 3 birds at a particular dose level of drug; both drugs were represented in 5 of the experiments.

† Percentage of erythrocytes parasitized.

‡ All treated chicks died of drug toxicity

Numerous asexual parasites were examined for evidence of drug-induced morphological or tinctorial changes. Owing to prolonged maintenance by blood-passage, the strain had lost most or all of its gametocytes; hence the effects on sexual stages were not determined. Both the cytoplasm and chromatin in parasites from quinine-treated chicks were abnormal, comparable to the descriptions of Hewitt and Richardson (1943).

The effects of azaserine were much more subtle than those of quinine. No effect could be detected on the pigment and cytoplasm. However, inhibition of chromatin division seemed to occur in the larger asexual parasites. Usually, the chromatin particles were diffuse, rather than compact, and poorly delineated from

one another. The maximum number seen in parasites from chicks treated with the higher doses of azaserine was only about one-half the number in the larger parasites from untreated chicks. Although further work would be needed for statistical documentation, it seemed that azaserine inhibited reproduction, at least in part, by reducing the number of merozoites per schizont. Evidence that azaserine interfered with cell division *in vitro* has been reported for *Escherichia coli* (Maxwell and Nickel, 1954) and *Corynebacterium diphtheriae* (David and Mudd, 1956). Moreover, Fernandes *et al* (1956) found that azaserine reduced the mitotic index of ascites tumor cells in mice.

Attempts to Reverse the Antimalarial Activity of Azaserine.

Encouragement for these trials and suggestions of materials to try stemmed from observations by others on the antagonism of the antimicrobial activity of azaserine *in vitro*. Antagonism of its growth-inhibition of *Escherichia coli* occurred with phenylalanine, tryptophan and tyrosine (Kaplan and Stock, 1954, or Maxwell and Nickel, 1954). Antagonism of its inhibition of purine synthesis by *E. coli* occurred particularly with all 3 of the above amino acids and to a lesser extent with glutamic acid, glutamine, and leucine (Gots and Gollub, 1956). Growth-inhibition of *Kloeckera brevis* was antagonized by alanine, arginine, histidine, and lysine (Reilly, 1954). Inhibition of maltase synthesis by *Saccharomyces cerevisiae* was completely prevented by leucine and partially by isoleucine, norleucine, phenylalanine, and methionine (Halvorson, 1954).

True competitive antagonism was not reported with any of these metabolites. It should be mentioned too that examination of all of the data in the foregoing papers suggests that azaserine acts through more than a single mechanism since the effects of potential antagonists varied with the test system. Maxwell and Nickel (1954) showed that tryptophan antagonizes only low levels of azaserine. Consequently, azaserine was given in only small amounts (0.01 to 0.03% in the diet) in the present antagonism studies.

Inasmuch as all attempts to antagonize antimalarial activity failed, only enough information to depict the scope of the trials is worth recording here. The following listing gives the name of the substances tested, their ratios to azaserine (arabic numbers), and the number of tests conducted on them (Roman numerals). Substances tested orally included adenine (40, I), dl-alanine (20, I; 40, II), l-arginine (20, I), dl-glutamine (40, I), glycine (40, II), guanine (20, II), l-histidine (10, I; 20, I; 40, I), hypoxanthine (10, I; 20, III), dl-isoleucine (40, III), l-leucine (20, I), dl-lysine (20, I; 40, II), dl-norleucine (20, I), parahydroxybenzoic acid (40, II), l-serine (10, I; 20, I), dl-threonine (40, I), thymine (20, V), l-tryptophan (20, I), l-tyrosine (20, I), uracil (20, I; 30, I), dl-valine (40, IV), and xanthine (20, IV; 40, I). Combinations of substances tested orally included adenine (20) and methionine (40, I), dl-alanine (40) and l-histidine (20, III), l-arginine (20) and dl-valine (40, I), and l-serine (20) and dl-lysine (40, II). Neither dl-glutamine (20, I) nor l-tyrosine (20, I) reversed the antimalarial activity of azaserine when all substances were given parenterally.

The preceding trials appear to be the only definitive attempts to reverse the activity of azaserine *in vivo* except for the work of Sternberg *et al* (1954) and Fernandes *et al* (1956); the former found that methionine failed to protect rats against

the hepatotoxic effects of the drug, and the latter that glutamine failed to alter the azaserine inhibition of glycine-incorporation into mouse tumor cells.

DISCUSSION

Interest in the antimalarial activity of a substance such as azaserine could stem from its potentialities as a practical antimalarial drug and its usefulness as a biochemical tool. Although the treatment regimen greatly influences the activity of antimalarials (Marshall and Dearborn, 1946) and not all possibilities have been exhausted, it seems unlikely on the basis of the present work that azaserine could compete with the better available drugs in the treatment of human malaria. Its failure to compare more favorably with quinine in the parenteral tests was disappointing in view of a relatively good comparison in drug-diet tests even though it is acid-labile (Fusari *et al,* 1954).

The antagonism tests represent an effort to determine the mode of action of azaserine. Each potential antagonist was tested under conditions favorable for *in vivo* reversal: high, sustained blood levels relative to the inhibitor. Such procedures were known to be adequate for demonstrating antagonism of beta-resorcylic acid by parahydroxybenzoic acid (Thompson *et al,* 1953a). Inasmuch as none of the substances tested reversed the effects of azaserine, it seems unlikely that any of them is directly involved in its mode of action against *P. lophurae* infections. The failure of various aromatic amino acids to antagonize the effects of azaserine against *P. lophurae* points to a fundamental difference between its antimalarial activity and its activities *in vitro* against bacteria or yeasts, which were notably antagonized by phenylalanine, tryptophan and tyrosine (supra). Since parahydroxybenzoic acid antagonized the effects of several simple substituted benzoic acids (Thompson *et al,* 1953a) but not the effects of azaserine, it would seem that these 2 types of substances exert their antimalarial effects through different mechanisms.

The fact that azaserine has both antineoplastic and antimalarial properties is of theoretical interest in providing another instance of correlation between these 2 types of activity. Although many antimalarials are inactive against neoplastic cells, an impressive proportion of the types of antineoplastic compounds are active against avian plasmodia. The antineoplastic activities of numerous nitrogen mustards and ethyleneimines (aziridine) have been reviewed by Stock (1954), and the antimalarial activities of several compounds of these types are given in Wiselogle (1946, Vol. II, pp. 257, 271, 363, 675). The 2,4-diaminopyrimidines are another example of compounds that are active against neoplasms (Stock, 1954) and plasmodia (Falco *et al,* 1951). Certain dihydrotriazines are active against tumors (Farber *et al,* 1953) and avian malarial parasites (Hewitt *et al,* 1954).* Unpublished work by the authors demonstrated feeble activity against *P. lophurae* in chicks by 2 other antineoplastic substances, namely, 6-mercaptopurine (Stock, 1954) and the antibiotic 6-diazo-5-oxo-1-norleucine (Burchenal and Dagg, 1956; Clarke *et al,* 1956). No basis for these correlations is readily apparent but the therapeutic indices in most instances would preclude the "concept of a general protoplasmic poison" as a satisfactory explanation.

SUMMARY

Azaserine had suppressive effect against blood-induced *Plasmodium lophurae* infections in chicks. It was compared with quinine by the drug-diet and intraperi-

toneal methods of treatment. In drug-diet tests, the 2 drugs had significantly different dose-response relationships; significantly less azaserine was required than quinine for low effect but more nearly equal amounts were required for high effect. Intraperitoneally, more azaserine was required than quinine at each level; the amounts required differed only slightly for low effect but substantially for high effect.

Twenty-two metabolites were tested for antagonism of the antimalarial activity of azaserine and none prevented its effect. Included among these were the various aromatic amino acids that antagonize the antibacterial and antifungal activity of azaserine *in vitro;* this indicates that its mode of antimalarial action differs from its effect on bacteria and yeasts. Evidence is presented that azaserine acts against plasmodia through mechanisms other than those responsible for the effects of the simple substituted benzoic acids.

The correlation between antineoplastic and antimalarial activity is reviewed.

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RESEARCH NOTE

RECORDS OF LARVAL *CONTRACAECUM* SP. IN 3 SPECIES OF MYSIDS FROM THE BRAS D'OR LAKES, NOVA SCOTIA, CANADA

Since 1945 the life history of *Porrocaecum decipiens*, the larvae of which infect the flesh of many marine fishes, has been studied by several scientists of the Fisheries Research Board of Canada. Scott (1950, Fish. Res. Bd. Canada, Atl. Prog. Rept. No. **48:** pp. 10–12) suggested that a crustacean might act as a first intermediate host of larval *P. decipiens*. Acting upon this suggestion, Dr. W. F. Black in 1951 and 1952 made extensive collections of invertebrates, principally crustaceans, from the salt water Bras d'Or Lakes, a region with an exceptionally high incidence of *P. decipiens*. In 1955, Black referred to the author for identification nematodes taken from 3 species of mysids. Three nematodes collected in the same area in October 1955 by Mr. D. H. Steele from the crustacean *Mysis mixta* were also sent to the author.

Fourteen nematodes from the mysids, *Erythrops erythrophthalma, Mysis mixta* and *Neomysis americana* were examined. All were larvae. Eleven, ranging in length from 2 to 17 mm, showed the characters of the parasitic genus *Contracaecum*. The remaining 3 were too small to permit generic identification.

Tattersall and Tattersall (1951, The British Mysidacea, Ray Society, London) recorded only 1 case of a parasitic worm, that of a cestode, in the Mysidacea. Accordingly, our records are apparently the first of larval *Contracaecum* sp. from the Mysidacea.

In view of the importance of mysids in the diet of many fishes, mysids probably serve as intermediate hosts of some of the species of *Contracaecum* which occur so abundantly in the digestive cavities of marine fishes.—D. M. SCOTT, *Fisheries Research Board of Canada, Biological Station, St. Andrews, N. B. and Department of Zoology, University of Western Ontario, London, Canada.*

THE TRYPANOCIDAL EFFECT OF NUCLEOCIDIN *IN VIVO*

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During the past few years the first reports have been made of antibiotics which are effective against trypanosomes *in vivo*. McCowan *et al* (1953) reported that erythromycin prevented death of mice infected with *Trypanosoma equiperdum*. Hewitt *et al* (1953) found that puromycin (Stylomycin) was effective against *T. equiperdum* and *T. cruzi*, although cures were not claimed for *T. cruzi*. Tobie (1954) showed that puromycin had a strong suppressive effect against *T. equiperdum, T. evansi, T. equinum, T. gambiense*, and *T. rhodesiense*. The aminonucleoside of puromycin was reported by Tobie and Highman (1956) to be somewhat effective against *T. congolense* and highly effective against *T. equiperdum, T. equinum, T. gambiense*, and *T. rhodesiense*. None of these reports dealt with a compound particularly active against *T. congolense*. More recently Hewitt *et al* (1956) reported that a new antibiotic nucleocidin was very active therapeutically against *T. equiperdum*. The physical characteristics and *in vitro* spectrum were described by Thomas *et al* (1956). In the present study we report the effective trypanocidal activity of nucleocidin, against *T. congolense, T. equinum*, and *T. gambiense*, three species representing three different groups of trypanosomes. As far as is known, this is the first antibiotic found to be highly active against *T. congolense*.

MATERIALS AND METHODS

The species used were *Trypanosoma gambiense*, Wellcome TS strain; *T. equinum*, Wellcome strain; *T. congolense*, Wellcome FN and Lumu strains. The Wellcome strains have been maintained in our laboratory since 1947. The Lumu strain of *T. congolense* was received in April, 1955 from the West African Institute for Trypanosomiasis Research through the courtesy of Dr. R. S. Desowitz. The drug was supplied by Lederle Laboratories. Data on dosage and toxicity were furnished by Dr. R. I. Hewitt.

Female Swiss white mice weighing approximately 20 grams and Osborn-Mendel white rats weighing approximately 200 grams were used as host animals. Techniques for inoculating and examining the animals were the same as previously reported (Tobie, 1954; Tobie and Highman, 1956). The number of trypanosomes used as inoculum was varied and is given in the table. In the first test one dose of 0.05 mg per kg of the drug was administered subcutaneously. In all other tests the regimen of treatment consisted of three daily doses of 0.5 mg per kg, a total of 0.15 mg per kg, administered in the same manner. Negative survivors, those apparently cured, were observed for 60 days, which is considerably beyond the period in which relapses have occurred in control or treated animals. These animals are therefore referred to as cured.

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EXPERIMENTAL DATA

The effect of nucleocidin against infections of *Trypanosoma congolense, T. equinum,* and *T. gambiense* in mice and *T. congolense* in rats is summarized in Table I. The drug was extremely active against *T. congolense* both in mice and

TABLE I.—*The effect of nucleocidin on trypanosome infections in rats and mice.*

Trypanosome species strain	*T. congolense* Lumu	*T. congolense* Wellcome	*T. equinum* Wellcome	*T. gambiense* Wellcome			
Host animal	rat	mouse	mouse	mouse			
No. of trypanosomes inoculated	1,000,000	10,000	1,000,000	100,000	10,000	10,000	1,000,000
No. of controls	10	10	20	19	10	14	10
Negative survivors	0	0	0	0	0	0	0
Total mg of drug per kg. of body weight	0.15	0.15	0.15	0.15	0.05	0.15	0.15
No. of daily doses	3	3	3	3	1	3	3
Rx started Day 1							
Parasitemia*	–	P	+	P	P	P	+
Negative survivors	4(1)**	10	10	10	10	10	9(1)ˣ
No. relapsed/no. inoculated	0/5	0/10	0/10	0/10	0/10	0/10	0/10
Rx started Day 2							
Parasitemia	+	P	++	++	+	+	++
Negative survivors	5	9	10	10	3	8	9(1)**
No. relapsed/no. inoculated	0/5	1/10	0/10	0/10	7/10	2/10	0/10
RX started Day 3							
Parasitemia	++	++	++	+++	++	+++	++++
Negative survivors	4(1)**	5	10	10	0	6	0
No. relapsed/no. inoculated	0/5	5/10	0/10	0/10	10/10	4/10	10/10
RX started Day 4							
Parasitemia	+++	+++	+++	+++			
Negative survivors	2(2)**	10	10	8(1)ˣ			
No. relapsed/no. inoculated	0/5(1)ˣ	0/10	0/10	1/10			
RX started Day 5							
Parasitemia	++++		++++	++++			
Negative survivors	0(5)ˣ		10	2(5)ˣ			
No. relapsed/no. inoculated	0/5		0/10	3/10			

* Parasitemia evaluated under 4 mm objective: P = 1–2 per drop; + = 1–2 in most every field; ++ = more numerous but could still be counted; +++ = too numerous to count; ++++ = extreme numbers prior to death.

** Cause of death not determined.

ˣ Died following treatment before drug could take effect or before treatment was begun.

rats, and against both the Wellcome and Lumu strains. In 2 tests no relapses occurred regardless of the day treatment was begun. The only failures encountered were in the third experiment, in which 6 of 40 mice relapsed. In all, 109 animals infected with *T. congolense* were treated. Of this number approximately 95% were cured. The drug also proved to be highly effective against *T. equinum.* Ninety-one percent of the mice infected with *T. equinum* in which treatment was completed were cured.

When administered as a single dose, 0.05 mg per kg cured *T. gambiense* infections in mice when treated on day 1; however, there were no negative survivors when treatment was delayed until day 3, at which time parasitemia was high. Three daily doses totaling 0.15 mg per kg were about twice as effective, curing 73% of the mice treated. When the number of trypanosomes used as inoculum was increased from 10,000 to 1,000,000 and treatment was not begun until day 3, again there were no negative survivors. Both the number of trypanosomes used for inoculum and the height of the parasitemia were apparently factors which decrease the chance of cure.

The compound had a very rapid action on the trypanosomes *in vivo.* Mice infected with *T. gambiense* which were moribund and in the terminal stage of the disease when treatment was begun, revived and parasitemia dropped rapidly, but relapse always occurred some days later.

The drug is reported (Hewitt, personal communication) to be highly toxic.

Some deaths of undetermined cause may have been due to toxicity. One of 5 non-infected rats treated with 3 daily doses of 0.05 mg per kg died the day after completion of treatment. The 4 survivors were sacrificed in apparent good health after 3 weeks. The drug may be more toxic for rats than mice. It is a possibility that 9 of the 25 rats infected and treated died because of the toxicity of the drug, while death of only 3 of 230 mice could have been caused by the toxicity. One must remember, however, that an infected animal may be more sensitive to the toxic effect of a substance than a non-infected animal.

SUMMARY

The trypanocidal activity of a new antibiotic, nucleocidin, against *Trypanosoma congolense, T. equinum,* and *T. gambiense,* 3 species representing 3 different groups of trypanosomes, is described. Three daily doses totaling 0.15 mg per kg produced approximately 95% cures in rats and mice infected with *T. congolense,* 91% in mice infected with *T. equinum,* and 73% in mice infected with *T. gambiense.*

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TRICHINELLA SPIRALIS INFECTIONS IN THIRY-VELLA FISTULATED RATS*

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A basic question concerning nutritional dependence of enteric parasites is which nutrients are obtained from the host and which from exogenous food in the lumen. Chandler, Read and Nicholas (1950) proved an endogenous thiamin source for the tapeworm *Hymenolepis diminuta* by parenteral injection into rats of radioactive vitamin. Earlier, by nutritional studies, Chandler (1943) proposed that the same tapeworm was independent of exogenous proteins and some vitamins, obtaining these essential nutrients by absorption from host mucosa.

Development of *Trichinella spiralis* outside the gut suggested that certain enteric factors are not necessary for maturation of infective larvae (McCoy, 1936; Mateff, 1941). Studies by Heller (1933) also supported the view that exogenous food may not be ingested during development while contact with enteric tissues is necessary for growth of larvae.

Fülleborn (1926) used the Thiry-Vella fistula to determine whether development of dog hookworm larvae (*Uncinaria stenocephala*) could occur in the intestinal tract without previous pulmonary migration. Twenty-one days after implantation of 900 larvae he failed to obtain hookworm eggs from fistular washings; later, however, he did observe hookworm larvae attached to the wall and found them to be undergoing morphological differentiation. Only 1/10 of the larvae implanted were recovered; he assumed that the majority had died and been digested. It was concluded that larvae in fistulas develop much farther, before they die, than they do in the lung of trachea.

Goodchild (1954), and Goodchild and Boodish (1955), reintroduced the Thiry-Vella fistula technique for *in vivo* nutritional studies of parasites. An excised section of the small intestine of white rats, free of food and with blood supply intact, was sewed to the flanks to make a fistula open to the surface at the 2 ends. Parasites were implanted into this isolated gut section free of exogenous food, while the host was maintained on a full and normal diet. *Hymenolepis diminuta* when placed in such a fistula has lived for as long as 4 days without exogenous food.

The objective of the present research was to determine whether *T. spiralis* larvae can establish themselves, grow, and reproduce normally when placed in fistulas totally free of exogenous food and certain digestive enzymes. This was accomplished by placing counted, artificially excysted larvae of *T. spiralis* in Thiry-Vella fistulas in white rats and tracing the subsequent course of infection.

MATERIALS AND METHODS

Thirty-five Wistar rats, 2 to 4 months in age, had their digestive tract surgically altered following the general method of Goodchild (1954). Some animals were starved for 12 hours prior to the operation to free the gut of food. Others were operated without having been starved. Rats were given 0.2 cc per 100 grams of

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body weight of 30% aqueous Veterinary Nembutal solution subcutaneously and for immediate anesthesia some were subjected to light ether anesthesia in addition. When anesthesia was complete they were clipped on both flanks from groin to thorax. The skin was sterilized with 70% ethanol and a 1-cm incision was made through the skin, musculature, and peritoneum on the right side. A section of the small intestine was pulled gently to the outside and spread out on the flank. The intestine was transected in 2 places, about 6 cm apart, in zones of minimal vascularization after application to the mesentery of cotton pellets soaked in 1 : 1000 epinephrin to cause vasoconstriction. Incisions were carried dorsally into the mesentery without injury to major blood vessels and without disrupting mesenteric support of the isolated piece. In order to maintain the excised piece in good condition it was kept moist with warm 0.8% saline or was shoved back into the abdominal cavity with only the severed ends exposed. The ends of the resected intestine were surgically rejoined by about 6 single sutures or by a continuous one; the rejoined gut was returned to the coelom. A second identical incision was made on the other flank. Fine forceps were introduced through the second incision and used to pull one end of the isolated gut section to the surface at the new opening. The ends of the fistula were sewed in place at the 2 openings by sutures. At the end of the operation the animal had a rejoined functional digestive tract, and an isolated piece of gut open at each end to the surface.

Immediately after the operation 0.1 cc of penicillin and dihydrostreptomycin-streptomycin was injected intraperitoneally to reduce the danger of infection. Water was offered to rats 8–24 hours after the operation and food after about 24–60 hours. After the fistular openings had healed they were flushed approximately every 3 to 7 days with warm 0.8% saline, animals being anesthetized with ether during the process.

Trichinella spiralis larvae, 4 weeks old, were obtained by standard peptic digestion of infected Wistar strain rat donors.

Four control animals, to be infected orally, were placed under light ether anesthesia. The excysted larvae were administered through a flexible, small-bore polyethylene stomach tube attached to a hypodermic syringe. Experimental hosts were also etherized during infection, and excysted larvae were placed deep into the fistula by means of a similar polyethylene tube and syringe. Washed larvae were suspended in saline only, and no other material was introduced into the fistula along with the worms. All fluid remaining at the openings was carefully removed by absorption with cotton and the area surrounding both openings was carefully dried.

Three different types of treatment after implantation were tried in 22 experimental hosts:

Series 1. In this series of 12 rats nothing further was done. The rats were returned to their cages after implantation of larvae. At least twice each week they were examined for signs of irritation around the openings. Although results from this series are considered significant, they are not conclusive because the rats did clean the openings and hence had opportunity for auto-oral infection by larvae or young adults escaping from the fistula.

Series 2. In this series of 6 rats the openings of the fistula were sewed tightly closed, covered with several layers of collodion, and finally covered from groin to axilla with several layers of 2-inch adhesive tape. Three rats in this

series succeeded in chewing through the adhesive tape and attempted to cleanse the fistular openings. Apparently the combination of sewing and collodion plus tight bandaging irritated the fistular zone; the skin became inflamed and ulcerated. For these reasons a new technique for the 3 rats which did not chew their taped flanks was devised. This was the same as the routine adopted for series 3 and will be explained there.

Series 3. After implantation of worms and thorough drying of the perifistular openings the areas surrounding the fistula were sprayed with several layers of a sterile liquid surgical dressing (Aeroplast, Aeroplast Corporation, Dayton 3, Ohio). The whole abdominal and thoracic area was then enclosed in a special girdle which prevented the rat from contacting either fistular opening. The base of the girdle was a piece of stainless steel 13 cm long, by 4.5 to 6 cm wide, and 0.45 mm thick. The edges and corners were rounded to reduce irritation. On the inner surface of the steel a layer of absorbent cotton was placed and over this and the whole surface were taped several layers of adhesive tape. The fistular openings were covered with 3 or 4 layers of 2-inch sterile gauze which encircled the body; over this were placed 2 pads of absorbent cotton directly over the fistular openings. The taped stainless steel girdle was then placed on the animal and made secure by additional strips of adhesive tape encircling the body and running anteroposteriad on the dorsal surface.

These animals were examined at least twice weekly and when needed the girdle was removed and the openings cleansed with 70% ethanol. Some slight skin irritation was evident in some but not in others. The irritated ones were treated with boric acid ointment and rebandaged with completely fresh materials. During the time bandages were being changed rats were prevented from licking any part of their body.

In addition to the 4 orally fed controls mentioned previously, 2 operated, orally infected animals, and 2 non-operated, non-fed rats were autopsied and processed with the same schedule as the implanted hosts. Animals were sacrificed from 4 to 5 weeks after challenge and the striated muscles were removed and digested with artificial gastric juice as above. Larval counts were made separately from diaphragm, appendages, and trunk muscles.

Infectivity of larvae recovered from experimental animals was tested by giving 200 orally to 2 control rats.

To study the growth and maturation of *T. spiralis* in the isolated gut section 13 rats were operated and had the usual Thiry-Vella fistulas made. Approximately 250 larvae were implanted into the fistula as before. Rats were sacrificed 1 to 15 days later, the fistula and the whole small intestine opened longitudinally and pinned out under saline. The total extent of the mucosa was carefully scrutinized with a stereoscopic microscope for nematodes either free in the lumen or buried in mucosa or muscularis. Parasites were fixed in hot glycerine-alcohol (9 parts 70% ethanol, 1 part glycerine) and allowed to stand uncovered until the alcohol had evaporated and the parasites were clear. They were mounted in glycerine jelly using the 2-coverglass method and lengths were determined from projected images.

OBSERVATIONS AND RESULTS

One hundred and sixty-one rats have been operated in these experiments and others. Ninety-nine (61.5%) of these survived the operation; 62 (38.5%) died before they could be used. Early post-surgical deaths seemed to be caused by shock or bacterial infection; later deaths (up to approximately 1 week) occurred because the rejoined main body of the intestine healed in such a way as to occlude the lumen. Food ingestion and passage of fecal pellets within 2 or 3 days after the operations were considered favorable signs.

Rats which survived and which ate and eliminated normally appeared normal with respect to coat color, texture, vigor, consumption of food and water, and fecal production. Fistulas either remained open, became occluded with dried blood, or eventually healed over completely. No difficulty was encountered with the first 2 conditions when the fistulas were flushed or worms implanted; in the last condition it was sometimes necessary to reopen the mouths of the fistulas surgically.

A string of odorless mucus emerged from the fistula when it was flushed. The material, often difficult to dislodge, particularly if 5 to 7 days elapsed between flushings, was tubular or flattened, solid, or non-uniform diameter, yellowish-white in color, and smooth externally.

Gross examination of skinned and eviscerated experimental animals revealed no abnormalities of any muscles, or evidence of unhealed lesions in the internal organs. The fistula possessed the normal, glistening pink intestinal appearance and usually was found to have been excised from the posterior jejunum or anterior ileum. The diminished diameter of the isolated section was similar to that of a normal intact starved intestine. After several weeks following the operation the amount of mucus produced seemed to diminish in amount. Adhesions of the isolated sections to the rejoined intestine were usually observed but these seemed to have little effect upon survival.

Microscopic examination, 4 to 5 weeks post-implantation, of selected bits of muscle revealed infections in each series of experiments in all rats challenged with larvae either through the Thiry-Vella fistula or orally. Rats not given *T. spiralis* larvae never revealed infections.

Actual counts of larvae in complete diaphragm presses, in some of both experimental and control series, agreed within 4–5% with counts of larvae after artificial digestion of the same diaphragm. The number of larvae was invariably higher in compressed counts than in digested counts.

Total numbers of larvae recovered by digestion from each of the 22 experimental animals of the 3 series ranged from 0 to 8,730. The number of larvae recovered by digestion from orally infected controls was always greater; diaphragm counts from orally fed controls ranged from 900 to 1400, while limb and body muscle counts all greatly exceeded 5,000.

Table I gives the numbers of larvae recovered from the various rats used in these experiments. In Series 1, in which parasites were implanted into the uncovered fistula, 200 or more larvae were used in the challenge dose. Parasites recovered ranged from 2 to 3189; counts from the body of 1 rat were not completed and hence the total for that rat is not given. Three other rats in this series had more than 200 larvae implanted; these revealed from about 1000 to 2300 larvae upon digestion. Six rats were used in Series 2, but 3 of these rats, as explained above,

possibly could have received an oral infection; they all revealed encysted larvae when sacrificed, but parasites were not counted. The 3 other rats in Series 2 never had opportunity to infect themselves orally; 3 days after larvae were implanted these 3 rats were placed in the metal girdle from which they were not removed until they were killed. Larvae from these 3 rats were counted. Unfortunately the larvae implanted into rats in Series 3 were defective; the thermostat of the incubator in which the donor rat carcass was digested failed and the temperature became elevated. These larvae tended to be straight and inactive at the time of implantation which generally indicates lessened vitality. Three of 4 experimental rats lacked larvae when examined. One rat had the usual larval load. One of 2 control rats

TABLE I.—*Number of* Trichinella spiralis *recovered by digestion method from each animal*

	Number of larvae given	Number of larvae recovered			
		Diaphragm	Limb	Body	Total
Series 1	200	19	38	17	74
	200	139	157	377	673
	200	41	86	212	339
	200	70	120	285	475
	200	115	165	245	525
	200	210	390	875	1,475
	200	2	0	0	2
	200	184	1,889	1,116	3,189
	200	17	93	—	—
	1,000	251	1,162	900	2,313
	3,000	46	774	201	1,021
	5,000	135	566	750	1,451
Series 2	200	630	2,600	5,500	8,730
	200	60	875	900	1,835
	200	215	1,520	2,492	4,227
	200 } 200 } 200 }	Not counted since hosts had a remote chance orally to contact the fistular opening.			
Series 3	200	700	750	481	1,931
	200	0	0	0	0
	200	0	0	0	0
	200	0	0	0	0
	Controls : fed orally				
	200	—	—	—	700
	200	—	—	—	11,435

fed orally with these same heat-inactivated larvae showed only 700 larvae in the total pooled musculature; the other control had only 11,435. These totals were much below the usual larval burden in orally challenged control rats.

In Table II are given the results of maturation of *T. spiralis* in the fistula. Ap-

TABLE II.—*Maturation of* Trichinella spiralis *in the Thiry-Vella fistula*

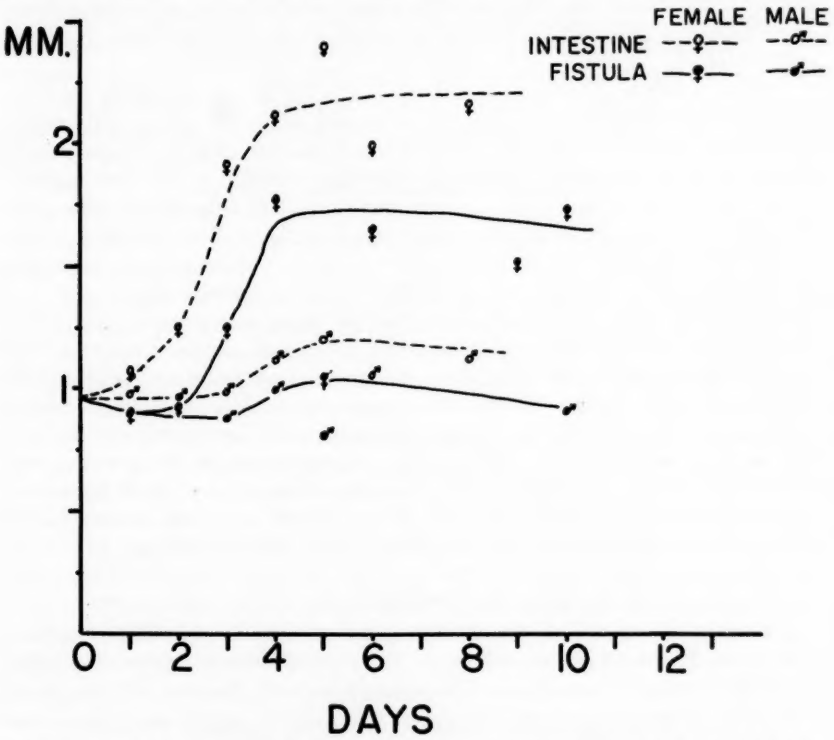
	Days of Development	Numbers Recovered*		
		In Fistula		In Intestine (all alive)
		Living	Dead	
Fistula open	1	2	0	0
	2	2	0	0
	3	2	0	2
	5	0	0	0
	6	15	0	0
	9	8	0	2
	13	0	0	1
	14	0	0	0
	15	0	0	0
Fistula closed	3	100	0	0
	4	50	0	0
	5	5	20	0
	10	15	5	0

* All rats received 250 larvae implanted into the fistula.

proximately 250 larvae were implanted, after which the fistula openings were left open (9 rats), or were encased in the stainless steel girdle (4 rats) as discussed above. In the open fistula series of 9 rats, 29 worms, all alive, were recovered. In this series oral autoinfection from the open fistula was proved since adult *Trichinella* were recovered from the intestine, 2 adults from any 1 host being the maximum. In the closed fistula of 4 rats during approximately the same period of time, 195 worms were recovered, of which 25 were dead. Oral infection in the metal girdle series was considered impossible; in support of this, no adults or juveniles were found in the intestine of any rat in this series.

Progressive development culminating in sexual maturity and copulation was attained in the fistula. Developing adults recovered from the fistula were found along its entire length. Many were threaded down into the superficial mucosa, others were free in the lumen, while still others were trapped in the luminal mucus. The latter were frequently dead and undergoing degenerative changes.

Growth of both sexes in the fistula was inferior to that in the intestine. In text-figure 1 is given a summary of average lengths of 421 adult *T. spiralis* recovered



Text Figure 1. Comparison of growth rates of male and female *Trichinella spiralis* in the intestine (dotted lines) and in the Thiry-Vella fistula (solid lines).

after varying periods from intestine and fistula. The length of 30-day excysted larvae, 0.97 mm, is exceeded within 1 day by intestinal females and within about 3

days by intestinal males. Females reached largest average size (2.4 mm) on the fourth day and retained this. Intestinal males reached their largest average size (1.2 mm) on the fifth day. Female worms from the fistula decreased in average size the first 2 days, during which time they averaged less in length than juveniles. During the next 2 days they grew rapidly to an average size of 1.75 mm, which they tended to retain. The experimental host sacrificed on the fifth day yielded substantially smaller worms than those from hosts on adjacent days. This fifth day host is considered an unexplained deviate. Males from fistulas exceeded juveniles in average length on only the fourth day (1.0 mm). In the fistula, adult worms thus failed to reach the average lengths of adults grown in the intestinal tract. Sexual maturation in the male occurred essentially without concomitant growth. A comparison of a 9-day fistular female (Fig. 1) and a 9-day intestinal female, (Fig. 2) is given on plate I. Recognizably dead parasites, milky-opaque, straight and motionless, were seen in some hosts, particularly the closed fistulas of 5-day and 10-day duration. Some of the other parasites when examined microscopically gave evidence of structural abnormalities, such as granulation of internal organs and dissolution of the stichosome, which were considered proof of actual or incipient death. These parasites were most frequently entrapped in the luminal mucus and were perhaps starving to death.

Although somatic growth of worms was less in the fistula than in the intestine, onset of sexual maturity was seemingly as prompt in the former as in the latter. Males were normal in appearance (Fig. 3) and function (Fig. 7); some females showed signs of insemination before the third day. Ovaries commenced egg production by the end of the first day and the uterus contained eggs not yet undergoing cleavage. Two-day-old females contained sperm in the seminal receptacle region of the uterus, but there was no evidence of beginning of cleavage; eggs were piled up single-file in the terminal portion of the uterus. Three-day old females contained cleaving eggs, but none had reached the vermiform embryo stage (Figs. 4 and 5). Four-day females and older (Figs. 5, 6, 7) all contained embryos in all stages of development (Fig. 8). After 10 days, normal males and females were still present in the fistula. In the open fistula series, adults were not recovered on the 13th, 14th, or 15th days. However, 1 adult was found in the intestine of a host sacrificed on the 13th day. These adult longevity results are in agreement with Christenson (1927), and McCoy (1932), who found intestinal adults in light infections for only 14 to 16 days, and with McCoy (1931) who found persistence of adult worms for less than about 14 days in previously immunized hosts.

DISCUSSION

Recovery of infective larvae from the musculature of experimental animals proves that *T. spiralis* implanted into the Thiry-Vella fistula is capable of completing its life cycle in an environment free of exogenous food, obtaining all its nutrients from host tissues or exudates. However, infections in experimental hosts were much lighter than in controls, suggesting that some factor favorable to sexual reproduction may be present in the intact gut, and is either missing or present in lesser amounts in the fistula. Ripe males and females removed from the fistula, however, seem packed to the normal limits with sperm and developing vermiform larvae. Ex-

cept for their diminutive size these worms look and behave exactly like adults reclaimed from the intact gut.

Growth is also less in the fistula than in the intact gut. Sexually mature males hardly exceed the lengths usually attributed to larvae. In effect then males become sexually mature without additional somatic growth. Females do grow slightly.

Obviously, physiological changes in the fistula such as altered pH and oxygen tension, absence of exogenous food, altered bacterial flora, presence of accumulated mucus, and other secretions or their lack may adversely affect maturation and reproduction of implanted larvae. Depressed infection rates may be caused by the death of all except the most robust larvae, *e. g.* only 29 (1.3%) of approximately 2,250 larvae were recovered in the open fistula series, and only 170 (17%) of 1,000 larvae were recovered in the closed fistula series. Undoubtedly some larval loss is accounted for by active migration of larvae out of the fistula; others were trapped in the luminal mucus where they died and disintegrated; others may have been trapped alive in mucus which peristaltically was brought to the surface and was cleansed by the rat or rubbed off in the cage. Depressed infection rates may also have been caused by suppression of larval production since females were considerably smaller in the fistula than in the gut; or they may be due to difficulties of larviposition in the fistular vascular system. Gravid females all showed the presence of sperm in the uterus; copulation apparently was not prevented and may have occurred early after implantation before the accumulation of mucus could interfere with free movement.

Except for one case, the closing of the mouths of the fistula resulted in a larger larval burden, with equivalent inocula, than leaving them open. This may be a reflection of the larger numbers of adults forced to remain in the fistula, and coincides with the data in Table II.

The operation itself seemed to exert no ill effect on the degree of infection since both operated and non-operated, orally infected controls carried the same larval burden. Larvae recovered from experimental hosts, infected via the fistula, also possessed full infectivity since such larvae fed orally to rats produced the same degree of infection as was found in controls.

Histological structure of worms grown to maturity in the fistula seemed completely normal. Except for decreased size the worms were completely typical.

Fistulated rats, too, appeared completely normal in every respect. The Thiry-Vella fistula has previously been shown to be a functioning unit of intestine in that vasopressor-free liver extract when introduced resulted in an increase of gastric secretion in about two hours (Kim and Ivy, 1933). Factors such as pH and oxygen tension may have been altered from larval and adult optima, but if controlled in subsequent work may help achieve higher infection rates. Changes in bacterial flora may also have been deleterious.

SUMMARY

1. *Trichinella spiralis* is able to live, mature, and reproduce in an intestinal region totally devoid of normal exogenous food.

2. Excysted juveniles established themselves in Thiry-Vella fistulas and succeeded in infecting most of 35 rats into each of which approximately 200 artificially excysted larvae had been implanted.

3. Fewer encysted larvae were recovered from skeletal muscles after fistular infections than from identical doses of larvae administered orally.

4. Some fistular adults had their anterior ends threaded into the mucosa, others were lying alive in the lumen in contact with the mucosa, while still others were trapped and dead in the copious luminal mucus.

5. Fistular adults were stunted in size as compared to intestinal adults, but nevertheless attained sexual maturity.

6. Encysted larvae, produced by fistular females, were normally infective for rats when administered orally and produced a normal muscular infection.

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Explanation of Plate

Figure 1. Sexually mature female from fistula. 9 days old.

Figure 2. Sexually mature female from intestine. 9 days old.

Figure 3. Sexually mature male from fistula. 4 days old.

Figure 4. Detail of terminal uterus, of fistular female, containing normal-appearing eggs. 3 days old.

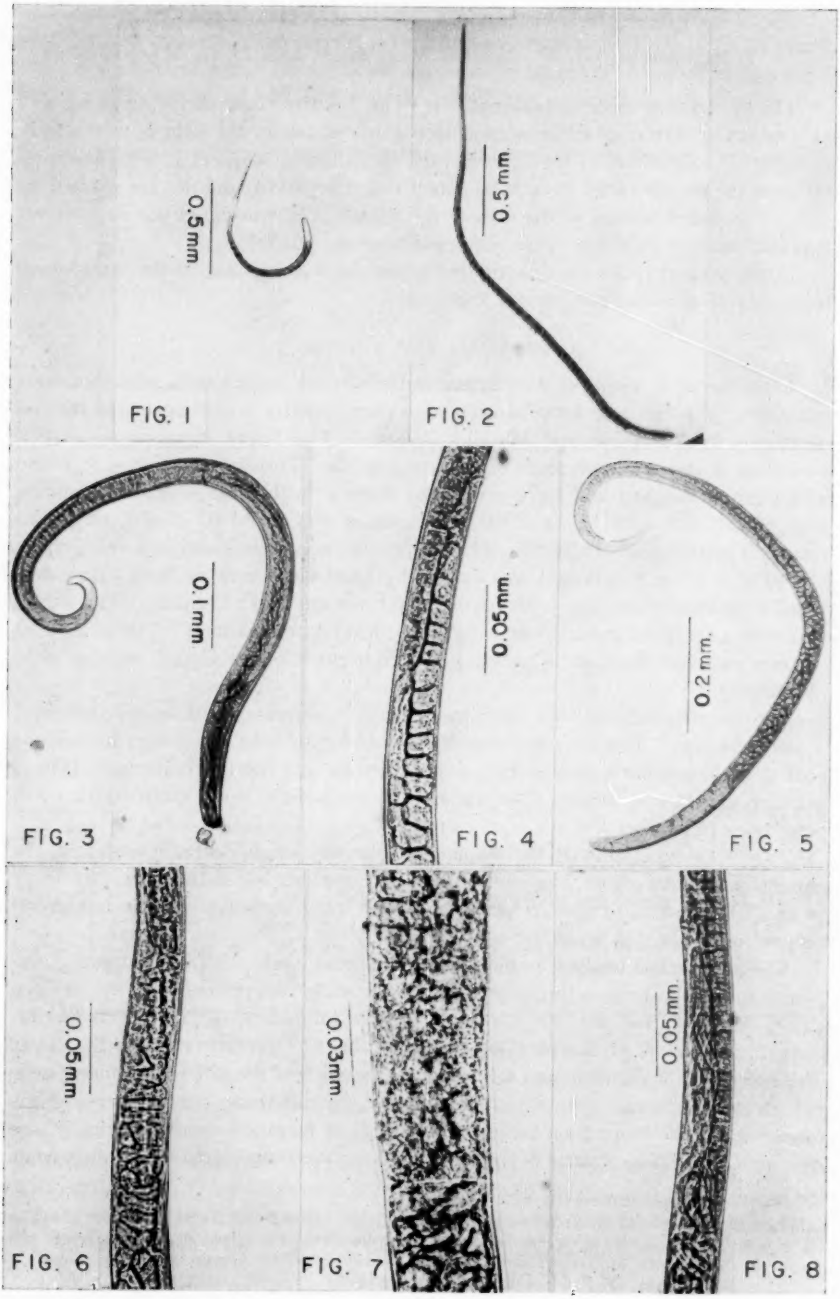
Figure 5. Sexually mature female from fistula. 3 days old.

Figure 6. Details of terminal uterus, of fistular female, containing cleaving eggs and near-vermiform embryos. 5 days old.

Figure 7. Detail of seminal receptacle region of uterus, of fistular female, revealing a mass of granular sperm. 5 days old.

Figure 8. Terminal uterus, of fistular female, with mature vermiform embryos. Vulva is conspicuous at top left. 6 days old.

PLATE I


0.5 mm
FIG. 1
0.5 mm
FIG. 2
0.1 mm
FIG. 3
0.05 mm
FIG. 4
0.2 mm.
FIG. 5
0.05 mm.
FIG. 6
0.03 mm.
FIG. 7
0.05 mm.
FIG. 8

QUANTITATIVE ASPECTS OF THE INTRADERMAL TEST WITH CERCARIAL ANTIGEN IN SCHISTOSOMIASIS

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The importance of the intradermal test as an auxiliary method for diagnosis and as a means of screening schistosome-infected individuals in the field is now widely recognized. Nevertheless the criteria used by different workers for the interpretation of the results varies to such an extent that comparative studies are made difficult. The determination of the area of the wheals was considered the most objective and accurate criterion (Pellegrino and Macedo, 1955b).

In the present paper the quantitative aspects of the response to the intradermal injections of cercarial antigen are discussed.

MATERIALS AND METHODS

Cercariae of *S. mansoni* shed by naturally infected *Australorbis glabratus* were concentrated in Büchner funnels fitted with porous plates according to the method developed by Pellegrino and Macedo (1955a). The heavy cercarial suspension was dried in vacuum in presence of calcium chloride. The dry material was ground in a mortar, weighed and transferred into a bottle with glass stopper containing glass beads. For each 10 mg of the powder there was added 10 ml of Coca's solution with merthiolate (1:5000). This suspension was maintained in a refrigerator (6° to 8° C) for 8 days and was shaken by hand about half an hour every day. Finally the suspension was centrifuged for 30 minutes at 1500 r.p.m.. The supernatant was decanted and used as antigen (1:1000 concentration). The other concentrations were obtained by diluting that concentration in Coca's solution with merthiolate.

The intradermal test was performed on 250 patients whose stools contained *S. mansoni* eggs. The patients were all males living in Belo Horizonte, an endemic area of schistosomiasis, the adults being policemen and the children pupils from a primary agricultural school. The majority of the patients were mulattoes, a small percentage of negroes and the rest white.

As it was expected that the response to the test would increase with age, the patients were taken from 3 age groups: 10 to 15 years (100 individuals); 16 to 19 years (50 individuals) and 20 years or more (100 individuals). The last group ranged from 20 to 49 years.

The intradermal test was performed on the flexor surface of the forearm. Individual tuberculin-type syringes were used for each concentration of the antigen (10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}) and for the control (Coca's solution with merthiolate 1:5000). A preliminary test injecting the 10^{-3} concentration in the upper and lower part of the right and left forearm showed that the difference among individuals was statistically significant but neither the differences among parts of the forearms nor the interaction individuals × parts of forearms were statistically significant. The different parts of the forearms were then considered for homogeneous

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reactivity. Each individual received all the injections on the same occasion according to the following procedure. Right forearm: upper, 10^{-3} concentration; lower, control. Left forearm: from proximal to distal, concentrations 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}. A distance of about 3 cm was left between the points of injections and a constant volume of 0.05 ml was administered for all the concentrations including the control.

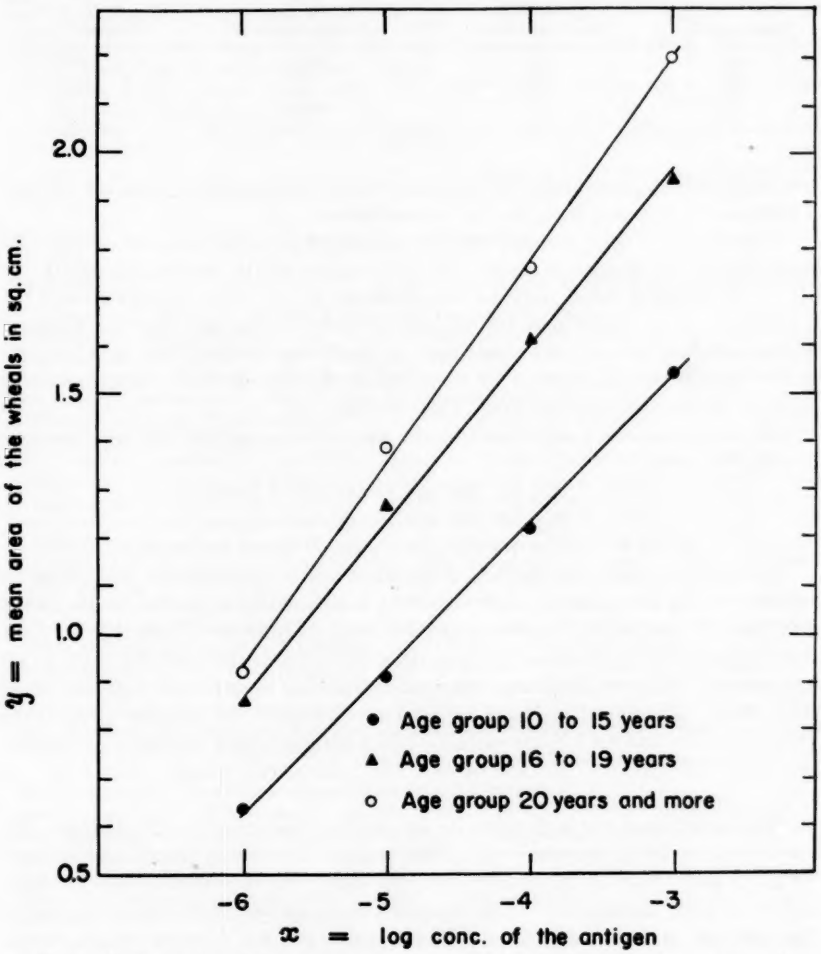

FIGURE 1: Regression of the mean area of the wheals (sq. cm) on log concentration of the antigen.

Fifteen minutes after the injections the limits of the wheals were outlined with ink and transferred to a slightly wet absorbent paper by pressing it over the wheals according to the method recommended by Martins (1949). The areas were measured with a planimeter and expressed in square centimeters up to the decimal fraction.

RESULTS AND DISCUSSION

The results are summarized in Table I. The average responses, i.e. the mean

TABLE I.—*Mean area of the wheals in sq. cm for the different age groups and concentrations of the antigen*

Concentrations	Age groups		
	10–15 years (100 individuals)	16–19 years (50 individuals)	20 years and more (100 individuals)
Control	0.57	0.62	0.64
10^{-7}	0.56	0.63	0.66
10^{-6}	0.63	0.85	0.92
10^{-5}	0.91	1.27	1.39
10^{-4}	1.22	1.61	1.76
10^{-3}	1.54	1.95	2.20

area of the wheals, increased with the concentration of the antigen in all age groups. There was no response from the 10^{-7} concentration.

Inspection of Figure I shows that the relation of the mean response to log concentration of the antigen is linear within the range of the concentrations 10^{-6} to 10^{-3}. The greatest mean response was observed in the oldest age group and the smallest in the youngest one, for all concentrations. The fact that the response to the intradermal test in schistosomiasis is smaller in children than in adults, already described by Martins (1949) and Pessôa & Barros (1953) became clearer with the determination of the area of the wheals.

The regression lines were fitted by the least squares method and the equations are the following:

$\hat{y} = 2.44 + 0.30\,x$ for the age group 10–15 years;
$\hat{y} = 3.04 + 0.36\,x$ for the age group 16–19 years;
$\hat{y} = 3.46 + 0.42\,x$ for the age group 20 years and more.

Analysis of variance for the data of the three age groups indicates that the mean response to log concentration (10^{-6} to 10^{-3}) is completely explained by the linear regression, F statistically significant at 0.1% level (Fisher and Yates, 1948). The mean square for the deviations of linearity is smaller than the ones for error in all age groups. Also the difference between individuals is statistically significant at 0.1% level. Therefore the linear regression will explain the relation between the mean responses and the log concentration of the antigen within the range considered.

SUMMARY AND CONCLUSIONS

The intradermal test with cercarial antigen was performed in 250 patients with stools positive for *S. mansoni* eggs. The patients were taken from 3 age groups: 10 to 15 years (100 individuals); 16 to 19 years (50 individuals) and 20 years and more (100 individuals). The injections were applied to the flexor surface of the forearms on the same occasion for each patient and five different concentrations of the antigen (10^{-7} to 10^{-3}) and a control were used. In all instances a volume of 0.05 ml was injected. Fifteen minutes after the injections the limits of the wheals were outlined with ink and the areas determined with a planimeter. The analysis of the results gave evidence of the following:

1—The average responses, i.e. the mean areas of the wheals, increase with the concentration of the antigen in all age groups. There was no response from the 10^{-7} concentration.

2—The relation of the mean response to log concentration of the antigen was linear within the range of the concentrations 10^{-6} to 10^{-3}.

3—The greatest response was observed in the oldest age group and the smallest in the youngest one, for all concentrations.

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RESEARCH NOTE

THE SURVIVAL OF *TRICHOMONAS GALLINARUM* IN CHICKEN DROPPINGS

Fresh cecal droppings containing numerous *Trichomonas gallinarum* were collected from experimentally infected chicks. These droppings were thoroughly mixed, and an equal amount of the material was placed in each of 6 small Petri dishes. The bottoms of 3 of these dishes had been covered previously with several thicknesses of moistened filter paper to prevent the fecal material placed thereon from drying; the bottoms of the other 3 dishes were not covered with moistened paper. Two of these dishes, one with the moistened paper and the other without, were subjected to a temperature of 6° C, two other similar ones to a room temperature of 18–25° C, and the remaining two to 37° C. Smears in physiological saline were made from the material in each dish at varying intervals and examined microscopically for trichomonads. When the smears from a particular dish were no longer positive for these flagellates, culture tubes containing Diamond's medium (Diamond, 1957, J. Parasitol. **43:**) were inoculated immediately with material from that dish. Subsequent inoculations were made at 24-hour intervals until a total of 5 tubes had been inoculated with the material in question. The following is a brief summary of the findings.

The presence of moistened filter paper in the Petri dishes apparently had no effect on length of survival of the trichomonads. As judged from the results of microscopic examinations of smears, trichomonads survived 7 hours but not 24 hours in droppings held at 37° C, 24 hours but not 32 hours at room temperature, and 72 hours but not 80 hours at 6° C. By means of the culture technique employed, it was determined that trichomonads survived 24 hours but not 48 hours in droppings kept at a temperature of 37° C and in those kept at room temperature. Cultures, inoculated with droppings kept in a temperature of 6° C for 120 hours, were positive for trichomonads; cultures made subsequent to that time were negative.

It has been reported (Richardson, 1934, Am. J. Hyg. **20:** 373–402) that trichomonad-infected droppings maintained at room temperature for 4 days and fed to 6 young chickens produced an infection of trichomonads in only 1 of them. The findings herein reported show, however, that under the experimental conditions involved, trichomonads failed to survive as long as 48 hours in droppings kept at room temperature. These parasites did, however, survive as long as 5 days in droppings exposed continuously to a temperature of 6° C.—D. K. MCLAUGHLIN, *Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.*

A HEMAGGLUTINATION TEST FOR TOXOPLASMOSIS

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The importance of adequate serological tests for toxoplasmosis is accentuated by the tremendous gains in knowledge concerning this infection, which stemmed from the description of the *in vitro* dye test by Sabin and Feldman in 1948. In the less than 8 years since this discovery, the serology of toxoplasmosis has been characterized and forms of the infection not previously known have been described and proved (Siim, 1951; Jacobs, Cook, and Wilder, 1954; Jacobs, Fair, and Bickerton, 1954). Together with the complement fixation tests, also developed by Sabin and his co-workers, the dye test offers the best means of diagnosis other than demonstration of the parasites. Dye test antibodies appear earlier than c.f. antibodies and persist longer; these differences, properly interpreted, have aided in the diagnosis of many clinical cases.

It is unfortunate, however, that the dye test is not as satisfactory from a standpoint of practice as it is technically. It requires live parasites, which are dangerous to handle, microscopic reading of the tests, and considerable care in most of the manipulations involved. Only a few laboratories in this country now perform the dye test, and it is unlikely that it will gain wider acceptance because of these difficulties. The same is true in other countries, where the additional difficulty is reported that normal serum for the necessary accessory factor substance is difficult to obtain (*fide* Desmonts).

Now, especially because the results of dye test studies have revealed and publicized various symptomatic forms of toxoplasmosis, the index of suspicion in fevers of undetermined origin, neonatal disease, and uveitis has been increased. The demand for the test by pediatricians, internists, and ophthalmologists exceeds existing facilities, and points up the need for a diagnostic procedure which can be practiced more generally. This need has stimulated explorations for various other means of serological testing.

The studies of Middlebrook and Dubos (1948), Boyden (1950–1951), Keogh *et al.* (1948), Alexander *et al.* (1950), Stavitsky (1954), and others have demonstrated the applicability of the hemagglutination test to a variety of antigens, both polysaccharide and protein in nature. The technique has already been tested in parasitic infections, such as schistosomiasis and trichinosis, by Kagan (1955–1956). In an effort to devise a test for toxoplasmosis which could be generally adopted, we have explored the applicability of this procedure. The following report presents preliminary results of these studies.

METHODS

Basically, the hemagglutination technique utilizes the phenomenon that red blood cells exposed to an antigen become sensitized to homologous antibody. Poly-

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saccharide antigens can effect this sensitization without previous treatment of the red cells; protein antigens can sensitize the cells after the latter have been treated with dilute tannic acid. It is necessary that test serums be absorbed first with non-sensitized red cells to remove any non-specific antibodies. Concentrations of red cells and antigens must be adjusted to give optimal titers. The cells of various species of animals have been used.

After preliminary explorations with rabbit erythrocytes and antigens prepared by lysing or by freezing and thawing toxoplasmas, and by the technique of Melcher (1953) for obtaining an acid-soluble protein fraction, the following procedure has been adopted for use in our laboratory.

Red cells are derived from sheep blood by bleeding the animal directly into a siliconized flask containing 1.2 volumes of modified Alsever's solution per volume of blood. The blood is kept in this solution until use, when the cells are washed 3 times with saline. One ml of packed cells is diluted with 30 to 50 ml of buffered saline at pH 7.2 to give an optimal concentration. The optimum will be discussed below.

Equal volumes of the diluted red cell suspension and of 1:20,000 tannic acid are incubated together in a water bath at 37° C for 15 minutes. Following this procedure, centrifugation, and washing in pH 7.2 saline, the erythrocytes are suspended in unbuffered saline and sensitized with antigen at pH 6.4, by mixing ingredients in the following order: 4 ml pH 6.4 buffered saline, 1 ml of antigen in saline, 1 ml of tannic acid-treated cells. The mixture is kept at room temperature for 15 minutes, the cells are separated by centrifugation, washed in 2 ml of 1:100 normal rabbit serum in saline, and resuspended in 1 ml of 1:100 normal rabbit serum.

All serums used in the test, i.e., the rabbit serum diluents and the test serums, are inactivated at 56° C for 30 minutes and absorbed overnight with equal volumes of washed packed red cells, to remove heterologous antibodies. The test serums are diluted in 1:50 normal rabbit serum.

The test is performed by adding 0.05 ml of sensitized red cells to 0.5 ml of each test serum dilution. The mixture is shaken, incubated for 2 hours at 37° C, read, shaken again, and allowed to stand overnight or longer at room temperature. The test tubes used are 12 × 75 mm. In one test, a tube with a smaller diameter was used, but this offered no advantage, and possibly a disadvantage in lessening the readability of results.

Antigens have usually been prepared by lysis of toxoplasmas in distilled water. Parasites are harvested from the peritoneal exudates of intraperitoneally infected mice into a tared tube containing saline buffered at pH 7.2. The harvested organisms are centrifuged and the supernatant discarded. Then the tube is weighed and a volume of sterile distilled water 10 times the weight of the sediment is added, the sediment is resuspended, and the mixture is allowed to stand at 5° C for at least 18 hours, with occasional shaking. The solid particles are then removed by centrifugation, and the supernate is adjusted to a concentration of 0.85% saline by addition of an equal volume of 1.7% saline. The antigen now constitutes approximately a 1:20 (wgt/volume) dilution of the original parasite harvest but contains only the water-soluble components of the parasites. It is stored in the deep freeze (−15° C) until used.

In some of the tests reported below, an occasional point differing from the above description may be indicated. This is because the procedure outlined was developed as experience with the test accumulated, and a few results pertinent to a particular discussion may be included despite a disparity in the technique.

Reading of the test is done mascroscopically. Characteristic patterns of hemagglutination form at the bottom of the tubes, which are read by means of a mirror. These patterns are graded + to 4+. At first, the system of grading used by Stavitsky (1954) was tried. However, it became obvious that the crinkling effect considered by Stavitsky as 4+ was not always found even with serums of high titer. Also, Stavitsky's + and doubtful grades corresponded with results obtained with completely negative serum or saline controls. In the latter case, a compact ring at the base of the tube may be the result of such physical factors as the smoothness of the base of the glass. The criteria described by Salk (1944) and by Charnock and Sabin (1953) appeared more realistic. Our criteria are shown in Figure 1. However, unlike the latter authors we have attempted to define grading up

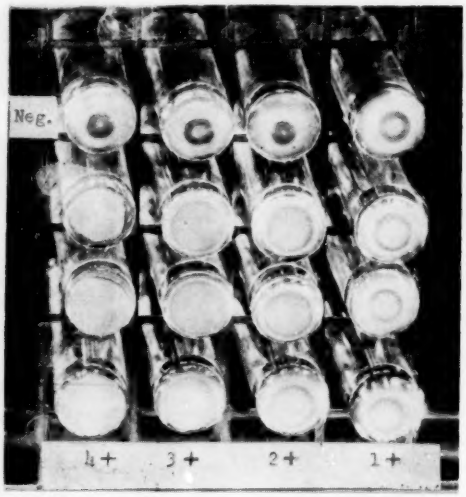

FIGURE. 1. Criteria for reading hemagglutination test. The top horizontal row represents negative results. The 4+, 3+, 2+, and 1+ readings are represented by 3 tubes each in the vertical rows above the numbers.

to 4+ rather than 2+. In all cases in which titers are reported below, they are based on a uniform progression from a strong to weak reaction and an endpoint of at least a 2+ hemagglutination. Thus, we are seeking a 50% endpoint. In the tubes showing little agglutination, it is sometimes difficult to make an accurate distinction between positivity and negativity. We have not yet selected our tubes for uniformity of the bottom, which may account for this, but because our endpoint is higher, we have no trouble reading positive reactions.

RESULTS

To date, 20 antigens have been prepared in the manner described above. Of these, 13 have been tested, a number of them in parallel runs to compare one against

others. The tests were performed with known positive human serums which gave steady dye test results. The motive of this testing was to determine the reproducibility of antigens rather than optimal concentrations of reagents. Only 1 of the antigens gave unsatisfactory results; it was tested after 2 months' storage at 4° C, and this prolonged storage without freezing may have been responsible for its loss of potency. All the other antigens gave satisfactory readings, although some showed higher potency than others. It has been noticed that those lots of antigen which gave the best results were derived from peritoneal exudates which contained very few white cells relative to numbers of toxoplasmas. On the other hand, antigens which yielded lower titers were derived from exudates with a somewhat larger proportion of white cells. However, at present this observation lacks quantitative support. From a practical standpoint, these variations in antigens indicate merely that each new lot should be tested against a reference antigen before it is put to routine use.

The variations between different lots of antigens are summarized in Table I, which presents the results obtained when they were tested against serums which

TABLE I — *Variations in titer obtained with different lots of antigen in separate trials*

Dye Test Titer of Serum	Number of Hemagglutination Tests, with Different Antigens, in which the Titer was 1:									
	4	8	16	32	64	128	256	512	1024	2048
1:16	4	4	2	1						
1:64			2	3	2	4	1			
1:256					1	2	2	3	1	
1:1024						2	2	3	2	1

TABLE II — *Agreement between dye test titers and hemagglutination titers in tests with different lots of erythrocyte-sensitizing antigens*

Dye Test Titer	No. of Tests	Hemagglutination Tests on Same Serums No. Agreeing Within 1 Tube	2 Tubes
1:16	11	7	11
1:64	12	9	12
1:256	9	7	9
1:1024	10	4	6

TABLE III — *Agreement between dye and hemagglutination tests of coded serums*

Dye Test Titer	Hemagglutination Titers Test 1	Test 2	Test 3
Negative	Neg.	Neg.	Neg.
1:16	1:16	1:16	Neg.
1:64	1:64	1:128	1:32
1:256	1:256	1:256	1:64
1:1024	1:2048	1:512	1:128

TABLE IV — *Time of demonstration of antibodies by different tests in a proven case of acquired toxoplasmosis*

Hospital Day	Dye Test	Hemagglutination	Complement Fixation*
1	Neg.	Neg.	Neg.
3	Neg.	Neg.	Neg.
5	1:64	Neg.	Neg.
6	1:256	Not done	Neg.
7	1:256	Not done	Neg.
8	1:1024	Not done	Neg.
9	1:1024	1:4	Neg.
10	1:1024	1:8	Neg.
12	1:4096	1:64	Neg.
13	1:4096	1:64	Not done
15	1:1024	1:64	1:32**

* The complement fixation tests were done by the Subsection on Immunology, Lab. Trop. Diseases.

** Routine complement fixation test results were negative here. A titer was revealed only by special techniques, (fide J. Bozicevich).

TABLE V — *Tests of the specificity of the hemagglutination test for toxoplasmosis*

Sensitizing Antigen	Antigen Tested for Inhibition	Titer* Obtained
Toxoplasma	—	1:2560
Mouse spleen	—	Neg.
Toxoplasma	Toxoplasma	Neg.
Toxoplasma	Besnoitia	1:1280
Toxoplasma	Mouse spleen	1:1280

* The serum had a dye test titer of 1:1024.

gave dye test titers of 1:16, 1:64, 1:256, and 1:1024. It should be noted that the same serums were not used in all tests; in some cases pools of serum were used, but in others, individual specimens of the desired titer were selected. All these trials were with human serum specimens, and with the antigens at the 1:20 dilution. Not all the antigens were tested against serums of all 4 titers.

While, obviously, some variation was found in the titers obtained with these antigens, nevertheless the tests all gave agreement in positivity of the specimens, even in those with low dye test titers. When the results of these tests are analyzed differently, to see how closely hemagglutination and dye test titers agreed, the findings are very encouraging. They are presented in Table II.

When it is remembered that our endpoints on the hemagglutination test readings were taken at 2+ or higher grades of agglutination, it appears obvious that the hemagglutination technique approaches the sensitivity of the dye test.

In order to rule out subjective influences in reading results on serums of known dye test titers, 3 tests were performed with coded serums. Five specimens of human serum were selected (different ones in each test), ranging in dye-test titer from negative to 1:1024. These were given different designations by a colleague, and then tested as unknowns in the hemagglutination procedure. The results were as presented in Table III. Again, good agreement was obtained between the tests. It is noteworthy that here, as in Table II, the greatest divergence of titers between the two tests was seen with the serums of highest dye test titer. However, the results in test 3 were uniformly lower than those in tests 1 and 2.

Since these results with the hemagglutination reaction showed generally satisfactory agreement with dye test titers, it was of interest to ascertain the ability of the test to reveal antibodies early in the course of an infection. For this purpose, serum specimens saved from a proven human case of lymphadenopatic toxoplasmosis were used. The case in question will be reported elsewhere (Kayhoe *et al*). It is known that the patient, a laboratory worker, pricked his finger with a *Toxoplasma*-infected needle 9 days before admission because of malaise and fever to the Clinical Center, NIH.

Specimens taken on hospital days 1, 3, and 5 were negative on the hemagglutination test. On hospital day 9, a titer of 1:4 was obtained, and on days 12, 13, and 15, titers of 1:64 were recorded. Comparative data on dye test and complement fixation test results on these same specimens are presented in Table IV.

In this comparison, it is seen that the hemagglutination test did not reveal antibodies as soon as the dye test, but did so sooner than the complement fixation test. It is unfortunate that specimens from the 6th, 7th, and 8th days were not available for testing by hemagglutination. Considering the closer agreement between dye and hemagglutination tests which has been found in some runs, it appears likely that it will be possible to improve the sensitivity of the hemagglutination test, by adjusting antigen and red cell concentrations to optimal levels, which was not done in the empirical system used here. These factors will be discussed below.

Further testing of this hemagglutination technique was devoted to studies on the specificity of antigens. This was tested in the experiment shown in Table V. In this case, a 1:100 lysed toxoplasma antigen was used to sensitize the red cells. In a parallel run, this antigen was compared with a 1:100 (wgt/vol) antigen prepared from mouse spleen by freezing and thawing. In other runs in the same test,

antigens prepared from mouse spleen, *Besnoitia jellisoni,* and *Toxoplasma* were added to the tubes containing *Toxoplasma*-sensitized cells and the test serum dilutions. These antigens were in a concentration of 1 : 10 (wgt/vol) for the two prepared from the parasites and 1 : 100 for the spleen preparation. They were added in 0.1-ml amounts to the serum dilutions before the addition of the sensitized cells. A human serum with a dye test titer of 1 : 1024 was used as the test serum, and another human serum, negative on the dye test, was used as the control.

The results were clear cut. The test with the *Toxoplasma*-sensitized cells gave a positive reaction at a titer of 1 : 2560. The test with the mouse spleen-sensitized cells gave a completely negative reaction. The tests in which the various antigens were added to the serum dilutions prior to addition of the sensitized cells gave the following results: *Toxoplasma* antigen—negative; *Besnoitia* antigen—1 : 1280; mouse spleen—1 :1280. Thus, *Toxoplasma* antigen specifically inhibited agglutination of *Toxoplasma*-sensitized cells; the other antigens did not.

All of the above studies were done with small lots of antigen, prepared from the peritoneal exudates of small numbers of mice. This was consistent with our purpose at that time, which was to see if a series of antigens could be prepared which would give reproducible results in the hemagglutination test, and to adopt a reliable system of grading. The red cell concentration used was close to that recommended by Stavitsky (1954). The density of the red cell suspension was measured in each case by lysing 1 ml of the diluted cell suspension with 5 ml of distilled water and measuring the optical density at 520 millimicrons in a Beckman spectrophotometer. It was found that the most usable concentrations of red cells gave readings varying from 0.4 to 0.6. In attempts to adjust the concentrations of both red cells and antigen, block tests have been performed using antigens varying in dilution from 1 : 20 to 1 : 500, and erythrocyte suspensions giving readings, by the above technique, of 0.4 to 0.6. In general, the results of these tests indicate that an antigen concentration of 1 : 200 and a red cell suspension giving a reading of 0.4 gave the highest titers with the test serum, with adequate negative controls. A reading of 0.4 is obtained with a suspension of approximately 2.0 to 2.5% cells, depending on the length of storage. Lower concentrations of cells gave less definitely negative readings in the controls. Higher dilutions of antigen (1 : 500) were found to diminish the titers obtained with positive serums. However, it appears that antigens vary in their initial potency and must be adjusted for optimal concentration prior to routine use.

DISCUSSION

It seems justifiable to conclude, from the above results, that reproducible antigens can be produced for the hemagglutination test, that a reliable system of grading is established, and that the hemagglutination reaction offers promise of providing a practicable serological procedure for the diagnosis of toxoplasmosis.

However, much remains to be accomplished before the hemagglutination test can be substituted for the *in vitro* dye test. Further work is necessary to compare the two tests in relation to their respective abilities to detect antibodies early in infection. Studies are necessary to ascertain whether or not they detect the same antibodies. In addition, some explanation must be found for the greater variations between hemagglutination and dye test results with serums of higher titer than with those of low titer. It is also necessary to determine the stability of antigens pre-

pared as described above, and to explore other methods, such as freezing and thawing or sonic disintegration, for the preparation of more potent antigens. The hemagglutination reaction must be tested against animal serums of various species, which may present individual problems.

On the whole, the results thus far obtained certainly indicate that these additional studies are worthwhile prosecuting. Given a standardized antigen and system, the hemagglutination test offers the prospect that it can be adapted for widespread use in routine laboratories.

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AN EXPEDITIOUS METHOD FOR COUNTING TRICHOSTRONGYLIDS AND OTHER HELMINTHS OF THE SMALL INTESTINE

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Studies in relations of helminths to their host frequently require a fairly accurate quantitative evaluation of the helminth burden. Data of this kind are also necessary in the conduct of screening programs designed to reveal a drug's anthelmintic efficacy.

On initiating a drug-screening program with the diminutive nematodes *Nippostrongylus muris* (Yokogawa, 1920) in rats and with *Nematospiroides dubius*

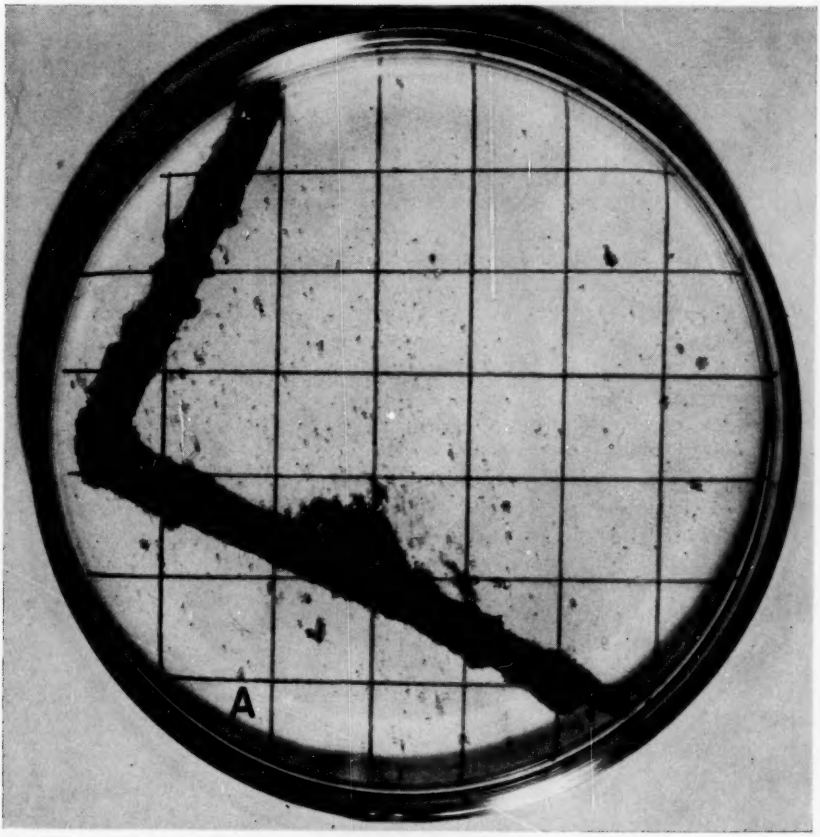

FIGURE. 1. Anterior segment of the small intestine of a mouse infected with *N. dubius*. The intestine has been turned inside-out to cover a wooden throat "swab" stick which has been bent to accommodate its length within the confines of a 9 cm. petri dish. The tissue has been covered with an acidified saline solution but the worms are still attached to the mucosa.

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Baylis, 1926 in mice, we found that the procedures conventionally employed to enumerate the worms residing in the intestine to be inordinately tedious and time-consuming when the number of animals in a series was adequate for statistical purposes. Not only do these worms tend to be tenaciously attached to the mucosa of the host's intestine and to become enshrouded in mucous secretions, but because of the spiral contours of the bodies of these worms which become entangled in copula, it becomes necessary to separate the worms for counting after slitting the intestine with scissors or scalpel.

Flushing the isolated but intact intestine with a pressurized stream of warm saline or even a strong detergent solution did not satisfy our needs either from the standpoints of accuracy and facility or economy of time.

After considerable experimentation along several lines, we came upon the simple method described below. This enables 2 technicians to isolate and count individually

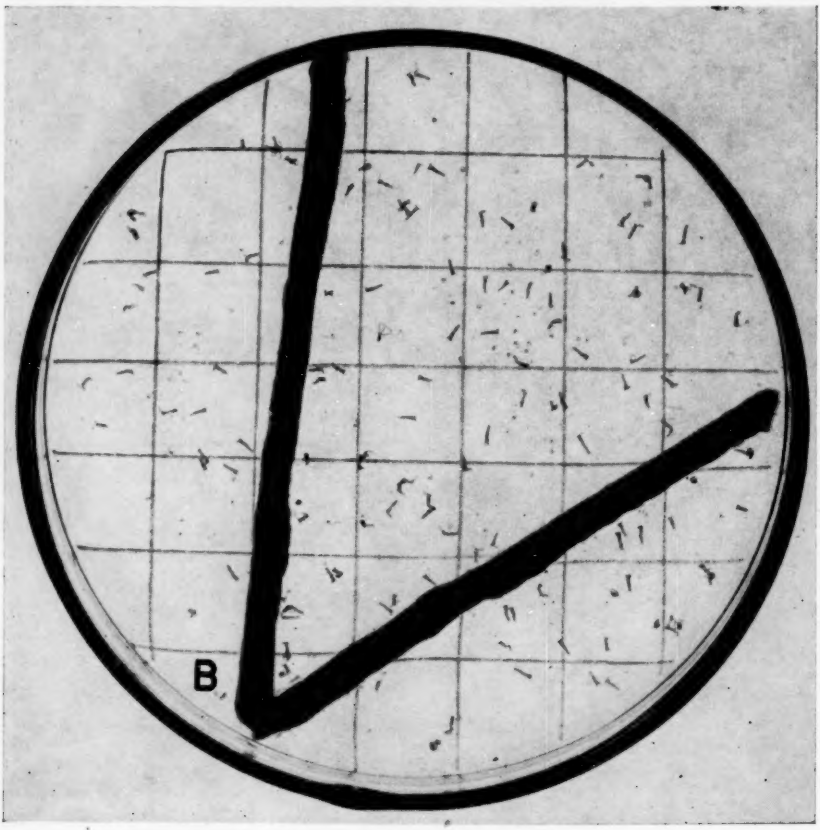

FIGURE 2. Ten minutes after the specimen was placed in a 45° C incubator. The entire worm population has abandoned its hold on the mucosa and can readily be dispersed over the entire field for enumeration. In this instance the small remaining quantity of intestinal debris has been pipetted off to render the worms more distinct in the photograph.

the worms (from 120 to 180 per mouse) harbored by 100 mice, infected with 200 filariform larvae of *Nematospiroides dubius,* in less than 6 hours without rush or sacrifice of reasonable accuracy.

Because of the novelty of the procedure, its utter simplicity and its probable applicability to other hosts infected with other types of worms (*e.g. Strongyloides* sp.) we deem the method worthy of being brought to the attention of others engaged in this field of work.

METHOD

Regardless of the technique adopted, the counting of worms is rendered more difficult by any food retained within the intestine. In order to eliminate as much obscuring debris as possible, the animals are deprived of all solid food for 24 hours before being sacrificed. We have found from extended observations that the normal habitat of the worms specifically involved in the present study is in the duodenum and proximal part of the jejunum. Consequently a segment of small intestine between 15 and 20 cm in length cut from a point slightly anterior to the pyloric sphincter may confidently be expected to contain all the worms with the possible exception of a straggler or two. Such a segment is removed by dissection and inverted along the length of an ordinary applicator stick (approximately 25 cm long and 2 mm in diameter) so that the mucosa is exteriorized.* The intestine with parasites still clinging tenaciously to its wall (see Figure 1) is transferred to a Petri dish and is covered with saline solution or tap water containing 1% HCl. The dish is transferred to a 45° C incubator for 5 or 10 minutes or until such time as it is convenient to start counting. Under the irritating influence of the heat and acidified ambient the worms are stimulated rapidly to disengage from copulation and to leave the mucosal tissue (see Figure 2). With the aid of a mounted needle the worms can be easily counted seriatim under the 3 × magnification of an illuminated colony counter (Dazor Mfg. Corp., St. Louis, Mo.) if the Petri dish is placed upon a square-lined white paper.

We have found the above-described method eminently suitable for establishing the intensity of infections with *Strongyloides ratti* whose size and distribution however call for higher magnification and the examination of a longer segment of the small intestine. The method can be used for counting tapeworms (which are frequently cut into pieces when an enterotome is used for slitting the intestine) and could be adapted for parasitic worms in such larger hosts as rabbits and sheep.

* This process is rapidly accomplished and is more simple to perform than to describe.

THE INCIDENCE AND DISTRIBUTION OF *HYDATIGERA TAENIAEFORMIS* AND OTHER INTESTINAL HELMINTHS IN SCOTTISH CATS

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Hydatigera taeniaeformis is the broadnecked tapeworm of the cat. The most extensively used synonym is *Taenia taeniaeformis* although another, *Taenia crassicollis,* is more common in the earlier literature. The larval form, frequently referred to as *Cysticercus fasciolaris,* is a strobilocercus found in the liver of rodents.

The larva has been studied by several workers because it may produce a sarcoma in the cyst wall surrounding it in the liver. Bullock and Curtis (1924) investigated the host-parasite relations; Miller (1930) and Campbell (1938) carried out extensive immunological studies on the strobilocercus. There are few reports, however, concerning the incidence of the adult tapeworm, other than that it has a world-wide distribution. Chandler (1925) recorded a 40% infection in 250 cats examined in Calcutta.

In the present paper are recorded the incidence and distribution of *H. taeniaeformis* and other intestinal cestodes and nematodes in Scottish city cats.

RESULTS

The estimation of the incidence of *H. taeniaeformis* was based on the direct examination of the small intestine of 1,707 stray city cats which had been killed by local authorities. This total is comprised of a series of random samples taken at monthly intervals from 1952 to 1955. Although this appears to be a large number, it is, in fact, only 2% of the total number of stray cats humanely destroyed in this period. The incidence of the other intestinal helminths is based on a more detailed examination which was carried out on 1,104 of the cats.

H. taeniaeformis

H. taeniaeformis was found in 132 cats giving an incidence of 7.7%. The number of cestodes per cat varied from 1–23; 47.7% harbored a solitary cestode, 25.7% harbored 2–3 cestodes while the remainder were infected with burdens varying from 4–23.

The distribution of *H. taeniaeformis* in the small intestine was influenced by the total number of worms present (Table I).

The stomachs of the stray cats were not examined but of 42 cats infected in the laboratory, 2 had *H. taeniaeformis* attached to the stomach walls. The complete strobila of these worms lay within the stomach but it is possible that they had been regurgitated from the small intestine. Grassi and Parona (1879) and Stiles (1894) described similar cases.

Dipylidium caninum

This was the commonest cestode and was found in 50.5% of 1,104 cats. Infections were usually heavy, frequently consisting of masses of more than a hun-

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dred worms. *D. caninum* invariably occurred far back in the intestine; in 96% of the infections, all the worms were in the posterior third. Even within this region, distribution was sharply delimited. The last 10 cm were always free of worms, presumably because of the lack of food.

Toxocara mystax

T. mystax was present in 13.7% of the cats examined. It was found in the first two thirds of the small intestine. It was also observed in the stomach of 3 out of 20 naturally infected laboratory cats which were examined within 10 minutes of being killed.

Toxascaris leonina

This nematode was recovered from the intestines of 15.3% of the cats. It had the same distribution as *T. mystax* but infections were usually heavier. A worm burden of 10–20 was common compared with 2–5 for *T. mystax*.

TABLE I — *The effect of increased worm burden on the distribution of* H. taeniaeformis *in the small intestine of 132 naturally infected cats*

No. of *H. taeniaeformis* Present Per Cat	*H. taeniaeformis* Present in the Small Intestine						Total No. of Worms
	Anterior Third		Middle Third		Posterior Third		
	a	b	a	b	a	b	
Light infection (1 - 3 worms)	13	9%	128	91%	0	0%	141
Medium infection (4 - 9 worms)	30	24%	91	73%	4	3%	125
Heavy infection (10 or over)	54	25%	146	68%	16	7%	216
Total	97	20%	365	76%	20	4%	482

a = Number of worms
b = Number of worms found in the region expressed as a percentage of the total recorded (last column)

Diphyllobothrium sp.

An unidentified species of *Diphyllobothrium* was found on 2 occasions. In each case the cestode was established at the beginning of the middle third of the small intestine. This genus has not previously been recovered from *Felis domesticus* in Great Britain although numerous cases have been reported from other parts of the world.

DISCUSSION

The low incidence of *H. taeniaeformis* compared with that of *D. caninum* is probably due to the less favorable conditions which exist in cities for the completion of its life-cycle. Whereas fleas, the intermediate host of *D. caninum*, remain common on city cats, extensive rodent control has reduced the chances of the *H. taeniaeformis* cycle being completed.

The predominance of solitary *H. taeniaeformis* in the cat may suggest the exist-

ence of an immunity due to premunition. However, this is improbable as superinfections have been established on many occasions in this laboratory and also by Miller (1932). It is more probable that this low level of infection in cats is due to the random catching of rats and mice which carry a single strobilocercus. This latter suggestion is supported by the observations of Luttermoser (1936) who found that the majority of wild rodents examined harbored only a single strobilocercus.

The distribution of *H. taeniaeformis* (Table I) indicates that these cestodes show a clear preference for the middle of the small intestine. This is particularly marked in the light infections where over 90% occurred in this region. The colonization of the anterior and, to a much less extent, posterior third is probably a result of population pressure. It is interesting to find that even with very heavy infections, worms are rarely found in the posterior part of the ileum, although this region often supports masses of *D. caninum*. This difference in the ecological distribution of these two cestodes suggests an underlying difference in their physiology.

Unfortunately, there is remarkably little information about the physical conditions or amounts of unabsorbed foodstuffs that are to be expected in the different regions of the intestine. Without such information, it is difficult to suggest the significance of this distribution. Archer and Hopkins (1957) record that, in rats, *Diphyllobothrium* sp. establishes itself in the posterior part of the ileum, in a position comparable to that occupied by *D. caninum* in cats, but does not grow until it migrates to a more anterior position. They conclude that the posterior part of the rat's ileum is too depleted to support the growth of *Diphyllobothrium*, although this region is commonly occupied by the small cestode *Hymenolepis fraterna*. It is possible that a similar difference in physiological adaptation exists in the case of *D. caninum* and *H. taeniaeformis*. All that can be concluded from the present results is that the distribution of the 2 species of cestode in the cat show sharp specific limits, the very existence of which indicates a response to an unidentified stimulus.

Concurrent infections with both *D. caninum* and *H. taeniaeformis* were observed on many occasions but, because of their different distribution, little interspecific competition is to be expected. *H. taeniaeformis* were rarely present in sufficient numbers to affect helminths farther down the intestine, and it is unlikely that cestodes occurring in the posterior part of the intestine would affect adults of another species occupying a more anterior region.

SUMMARY

The incidence of cestodes and nematodes in the small intestine of stray cats living in Scottish cities has been estimated to be: *Hydatigera taeniaeformis*, 7.7% *Dipylidium caninum*, 50.6%; *Toxocara mystax*, 13.7%; and *Toxascaris leonina*, 15.3%. The distribution and intensity of infection of these helminths within the gut is recorded and their inter-relations discussed.

ACKNOWLEDGMENTS

I am extremely grateful to Dr. C. A. Hopkins for his criticism and many helpful suggestions during the course of this work.

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RESEARCH NOTE

AGE OF HOST AND ROUTE OF ADMINISTRATION AS FACTORS INFLUENCING THE SUSCEPTIBILITY OF TURKEYS TO *TRICHOMONAS GALLINARUM*

Nine 1-week-old trichomonad-free poults were divided into 2 groups of 4 and 5 birds, respectively. Nine 9-week-old trichomonad-free poults were divided into similar groups. The individual poults of the various groups were inoculated with approximately 1,000,000 bacteria-free *Trichomonas gallinarum* of strain DT-79, which had been isolated by the author in October, 1955, and subsequently maintained in culture by Mr. L. S. Diamond (1957, J. Parasitol. **43:** in press). Five of the younger poults and 5 of the older birds were infected *per anum*. The 4 remaining birds of each age were infected *per os*.

Four days following inoculation, trichomonads appeared in the droppings of all of the younger birds, and in the feces of the older poults which had received the flagellates *per anum*. Droppings of the older birds which had been inoculated *per os* remained negative for these parasites.

All of the birds were autopsied on the 6th day following inoculation. Smears of cecal contents revealed the presence of large numbers of trichomonads in the case of all of the younger birds. Only small numbers of these parasites were found in the cecal contents of the older birds that had been inoculated rectally. Trichomonads were not found in the cecal contents of the older birds that had been inoculated orally, and cultures inoculated with material from the ceca of these birds were negative also.

The findings show that the older birds were less susceptible to *T. gallinarum* than were the younger ones. Consequently, for experimental purposes, rectal inoculation of these organisms may be more certain to produce an infection in birds 9 weeks of age than is inoculation by the oral route.—D. K. MCLOUGHLIN, *Animal Disease and Parasite Research Division, Agricultural Research Serivce, U. S. Department of Agriculture, Beltsville, Maryland.*

PHYSICAL OBSERVATIONS ON *ECHINOCOCCUS* EGGS, PART II: A SIMPLE METHOD FOR THE DETERMINATION OF VIABILITY

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Infection of man with hydatid disease in several rural areas of Alaska has resulted from contaminated food and water supplies and general insanitary conditions. Investigations are at present being conducted to ascertain methods of rendering the eggs of *Echinococcus spp.* non-viable by chemical means without destroying the palatability of food and water. One of the principal difficulties in this work has been the lack of a simple method for determining viability of the eggs following treatment. The red-backed vole, *Clethrionomys rutilus dawsoni* (Merriam) is a natural intermediate host of *Echinococcus multilocularis* Leuckart, 1863, in Alaska, and also serves as a bio-assay animal; however, the use of animals for determining viability presents several problems. The red-backed voles must be trapped continuously and a colony maintained. This is often difficult and always time-consuming, especially in the winter months. After administering the eggs to the voles a waiting period of from 5 to 15 days is necessary before the viability of the eggs may be ascertained. It is also possible that a previous infection by other helminths may interfere with infection by *Echinococcus* larvae, even though the eggs are viable.

The present study describes a method of artificially liberating live oncospheres from the embryophore. The literature has been reviewed rather thoroughly by Silverman (1954) who was concerned with *Taenia saginata*. Evidently the only other attempt at liberating oncospheres from *Echinococcus* sp. eggs was made by Ross (1929). It is probable that the described procedure may be applied to the eggs of other cestodes, although this has not been attempted.

METHODS

Several methods for determining viability of *Echinococcus* eggs were considered. Liberation of the oncospheres in an artificial medium was considered advantageous, however, since it would leave no doubt concerning viability. Too, the final procedure is relatively simple and requires approximately 20 minutes to run.

The original cestodes used in the present experiment were obtained from arctic foxes, one of the final hosts for *Echinococcus multilocularis*. The cestodes were fed to red-backed voles which in turn were fed to dogs after the larvae became infective. When *Echinococcus* eggs were required for an experiment a dog was autopsied, the worms removed from the intestine, and maintained in tap water at 5° C. Apparently none of the eggs lost their viability under these conditions.

Two solutions are required for liberation of the *Echinococcus* oncospheres. Solution no. 1 is prepared by adding 0.10 g pepsin 1 : 3000, 2.4 g NaCl and 0.057 g $Na_3PO_4 \cdot 12H_2O$ to 300 ml 6.7×10^{-3}M HCl. This solution is 5×10^{-4}M with respect to phosphate and 0.8% with respect to NaCl. Solution no. 2 is prepared by adding 1.20 g trypsin 1 : 250 and 2.4 g NaCl to 300 ml 6.775×10^{-2}M Na_2HPO_4.

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* Chemist.

The solutions have been found to maintain stability for several months if kept at 5° C.

Five ml of each solution are measured into separate 15-ml tubes which are placed in a constant temperature bath at approximately 38.0° C. When the tubes have had sufficient time to come to the designated temperature, 1 ml of the solution containing *Echinococcus* eggs, usually in the gravid segments, is placed in the tube containing the pepsin solution. An incubation period of from 3 to 5 minutes is allowed, before addition of the trypsin solution. After addition of the trypsin solution, approximately 30 seconds is allowed for eggs and free oncospheres to settle. Approximately 1 ml of solution is removed from the bottom of the tube with a rubber-bulb pipette and placed in a Sedgwick-Rafter slide. Upon microscopic examination, moving oncospheres may be observed at 100× if the eggs are viable.

DISCUSSION

The addition of an activator, such as sodium phosphate, to the pepsin solution was found to be essential. Sodium phosphate is by no means specific to this reaction, however, since it was found that any one of 20 compounds tried will perform the same task to varying degrees. Among the better activators used were glycine, ethylenediaminetetraacetate, diethyldithiocarbamate and urea. The evidence indicates that the activator operates as a complexing agent in binding traces of heavy metals. The fact that diethyldithiocarbamate, which is specific for copper, activates this reaction lends authority to this hypothesis.

It was found that neither the time nor temperature of incubation are critical. Live oncospheres were obtained with incubation times varying from ½ to 10 minutes and with temperatures varying from 35° to 40° C. An incubation time of 5 minutes and a temperatures of 38° C seems to be optimum for this system, however.

CONCLUSIONS

The described method affords a fast, relatively simple means for determining viability of specific cestode eggs. The results are positive and eliminate the necessity of infecting susceptible mammalian intermediate hosts. This method does not take into consideration the possibility of the oncospheres being viable, but damaged to the extent that they are no longer infective.

ACKNOWLEDGMENT

The writer is indebted to Dr. Laurence Irving, Dr. Robert Rausch and Dr. Edward Scott, Arctic Health Research Center, for their many helpful suggestions and criticisms during this investigation.

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PIGMENTATION IN THE FOOT OF *LITTORINA LITTOREA* AS A MEANS OF RECOGNITION OF INFECTION WITH TREMATODE LARVAE*

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Littorina littorea serves as the intermediate host in the life history of *Cryptocotyle lingua,* a trematode from the intestine of marine birds. In response to the need for infected snails for experimental purposes, an attempt was made to discover some means of distinguishing infected from uninfected animals without use of the isolation method, by which snails are kept for several days in separate containers and the water examined periodically for emerged cercariae.

The isolation method has several disadvantages. It is too time-consuming, the snails show a high mortality rate, and some snails, even though infected with mature larvae, will not shed cercariae during isolation for several days. Furthermore, the isolation method fails to identify infected snails in which the larvae are not yet mature.

It was observed that the foot of an infected snail (*Littorina littorea*) becomes dark yellow to brown in color while the foot of uninfected specimens remains white. The color of the expanded foot can readily be observed through the glass as the animal crawls up the sidewall of a dish. To identify infected specimens rapidly, collections of snails are placed in sea water in tall glass containers such as battery jars or large beakers. The infected individuals can now be recognized by the yellow to brown foot color and the specimens sorted into infected and uninfected categories. The method has been used with 5 different collections of *Littorina* during the months of June, July, August, and November, and has proved uniformly successful in separating infected from uninfected animals.

Unless a snail has a distinctly brown foot, it is not infected. This observation was carefully checked by crushing and examining all the specimens, following sorting into brown- and white-footed groups. In one experiment, 82 snails, collected from a heavily infested area, were assorted into 2 classes, 60 with white foot and 22 with brown foot. Examination of the specimens after crushing showed no infections among the 60 with white foot, whereas 21 of the 22 with brown feet were infected.

In a test involving 150 snails, 8 brown-footed individuals were all infected and the remaining 142, none of which had a pigmented foot, showed no infections when crushed and examined. In November of 1955, from 200 *Littorina littorea,* 15 infected specimens were identified by the brown foot color, and in August, 1956, 20 infected specimens were separated in 2 hours from a collection of 400 snails. Examination after crushing of about half of the white-footed specimens yielded no infections and the rest were discarded.

These results show clearly that unless a snail has a brown-colored foot it is not infected. Among the infected specimens, 2 other trematode species in addition to

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* The authors acknowledge the assistance, in certain of these experiments, of Mr. Sylvan Nass.

Cryptocotyle lingua were encountered, and in each case the foot of the host showed the characteristic dark pigment. Some infections were in early stages which would not have been detected by isolation techniques, since no mature cercariae appeared in the crushed specimens while rediae and immature stages of cercariae were found in profusion.

It was a matter of interest to discover the source and mechanism of the pigmentation which accompanies infection. As a first step, the foot of 4 heavily infected snails was removed and cleaned of surrounding black tissue. The cleaned tissue was then suspended in 0.5 M KCl and homogenized in a Ten Broeck tissue grinder. The resulting brei was centrifuged in a Spinco Model L preparative ultracentrifuge at 100,000 × g (ave.) for 30 minutes. The supernatant fluid after sedimentation of solids was water-clear and contained none of the pigment, whereas the pellet was brown. In subsequent tests with other aqueous solvents, we confirmed the observation that the pigment in question is entirely insoluble in water, or is at least bound to structures which cannot be dispersed in aqueous solvents.

The supernatant from the first sedimentation was discarded and the brown pellet resuspended in absolute ethanol. The suspension medium immediately took up the color, and upon centrifugation, remained yellow while the precipitate of denatured protein and tissue debris was white. Thus the pigment is alcohol-soluble, and can be prepared free of protein by means of alcohol extraction of the pigmented tissue.

For control purposes, a quantity of foot tissue roughly equal to that obtained from the infected snails was dissected from 4 normal (white-footed) animals, and the procedure outlined above repeated. Absorption spectra in the region 350 to 750

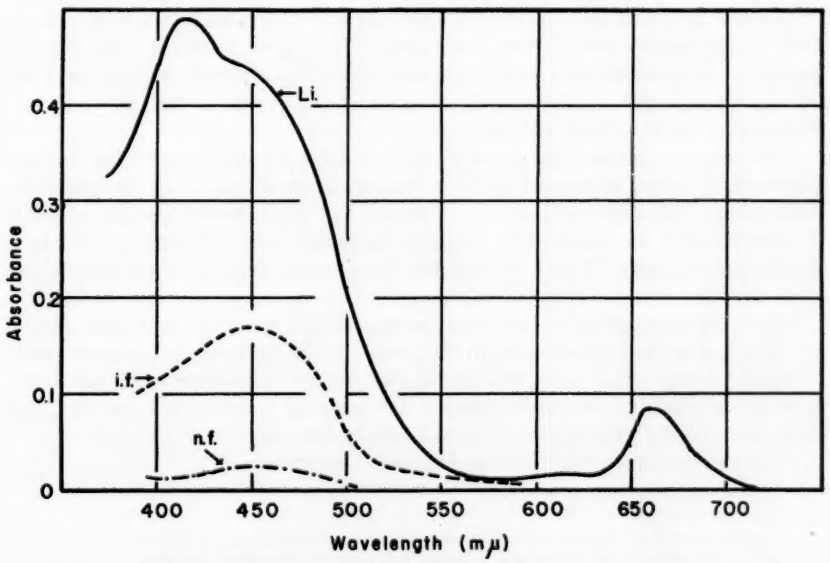

FIGURE 1. Absorption spectra of alcoholic extracts of tissues of *Littorina littorea*. Li., = liver extract, i.f., = extract of infected foot, n.f., = extract of non-infected foot. Spectra measured with modified B & L Spectronic Colorimeter.

millimicrons were made on both preparations using an alcohol blank. The spectrophotometer was a Bausch and Lomb "Spectronic 20" instrument equipped with a special revolving double cell-water cooler sold by Arthur H. Thomas Co. of Philadelphia. Portions of these spectra are shown in Figure 1, with the spectrum of the infected material designated "i.f." and that of the control extract labelled "n.f.".

It is clear from these spectra that the infected tissue bears a pigment which is present in only a negligible quantity in the normal foot tissue. The pigment has an absorption peak near 450 millimicrons. A second, smaller peak is present at about 660 millimicrons, but this is not plotted in the figure.

Infected animals were next removed from their shells and examined for a possible source of the pigment. The "liver" was an immediate candidate, since this organ is, in normal animals, deeply pigmented (brown and orange) and is, in infected animals, partially or wholly destroyed by the burrowing of the parasitic larvae. Closer examination revealed that the normal liver contains regions of intense orange color present in the form of coiled strands which invest the surface just beneath the body wall. We reasoned, therefore, that the pigment in the foot of infected animals must be released through the destruction of liver tissue and thence carried down, probably by the circulation, into the foot muscle.

This hypothesis was easily tested. Several normal livers were crushed in ethanol and the pigment extracted. The extracts were adjusted with respect to concentration, and studied in the spectrophometer against ethanol blanks. One such spectrum is shown in Figure 1, marked "Li". The spectrum shown is for the pigment in 95% ethanol. The material manifests spectral characteristics identical with those of the pigment extracted from the infected foot tissue, i.e., absorption peaks at 450 and 660 millimicrons (and hence, of course, corresponding to a yellow "color") with a minimum at 560–630 millimicrons. There is, in addition, a sharp peak at 420 millimicrons in the liver extract. This peak is absent from the foot pigment. It probably represents hemochromogen-like material or some degradation product of dietary chlorophyll present in bound form in the liver cells and hence extracted therefrom with ethanol.

The yellow pigment from *Littorina littorea* livers resembles, spectrally, the bile pigments, or bilins, of mammalian liver, but can be shown not to be on open-chain tetrapyrole. The pigment is soluble in alcohol, but not in water, and not in alkali. It does not give the Gmelin test, which is diagnostic for bilins (see, for example, Lemberg and Legge, 1949). In addition, the pigment does not form colored zinc salts.

Since the pigment is prepared easily in considerable quantity, it was not difficult to identify it at least with respect to the groups of the lipid-soluble pigments found in animal tissues. It is to be understood that without chromatographic separation, it is not possible to determine whether the material consists of more than one molecular species, although this is most probably the case.

The pigment shows the following diagnostic characters:

Yellow to orange color in the tissue
Insoluble in water
Soluble in various organic solvents, following denaturation of tissue protein
Yellow in ethanol and chloroform solution, but rose-red in carbon disulfide
Chloroform solution rendered blue on mixing with concentrated H_2SO_4. Pigment soluble in concentrated mineral acids and in glacial acetic, and blue or green in sulfuric, depending on pigment concentration

Dissolved in CS_2, the pigment shows a broad absorption band in the visible region of the spectrum.

These characters establish the pigment(s), according to Fox (1953), as carotenoid. The presence of such a pigment in *Littorina* digestive organ is in no way surprising, for there is a considerable literature describing carotenoids in various molluscs (Fox, 1953).

SUMMARY

Specimens of *Littorina littorea* infected with trematode larvae can be positively identified and separated from non-infected animals by inspection of the expanded foot. In infected animals, this assumes an orange to brown color, in contrast to the white or grey color of the foot in normal specimens. The pigmentation observed is a result of destruction of "liver" tissue by the parasitic larvae, leading to a release of a yellow-orange pigment into the circulation. This pigment, normally confined to the digestive organ, is a carotenoid whose ethanol solution shows absorption peaks at 420, 450, and 660 millimicrons.

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A RODENT ECTOPARASITE SURVEY OF HAIFA PORT

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The port of Haifa was completed in 1932, and rodent and rodent ectoparasite surveys were undertaken from 1933 onwards, although their results were never published. The importance of these surveys was demonstrated when plague-infected rats were found in the port in May 1941 followed some months afterwards by 15 cases of human plague around the port. Plague-infected rats continued to be found until January 1943. All rats trapped were negative for plague from this date until June 1944 when infected rats once again appeared in the port; this was closely followed by an epidemic of 29 human cases with 10 deaths among port workers and in the surrounding town (Btesh, 1945). In July 1947 plague-infected rats were again found in the port. This had been preceded by the appearance of 1 human case of bubonic plague on June 26, and during the month of July, 15 additional cases appeared in the town, until the anti-plague work had its effect. This epidemic was one of the earliest to be controlled by the widespread use of D.D.T., although in this case, the major part of the work was done with a 5% D.D.T. spray, rather than with the now more conventional use of a 10% D.D.T. dust (MacKenzie-Pollock, 1948).

With the outbreak of civil disturbances and hostilities in Palestine in 1947–1948, the rodent ectoparasite surveys ceased, and were not again commenced until January 1954. This report summarizes the data of that survey, from January 1954 until July 1955.

Rat-trapping was done throughout the port in warehouses, restaurants, offices etc.; baited, cage-type traps were used, the rats being transferred alive into cloth bags and then chloroformed and combed in the laboratory. They were then dissected and examined for any signs of plague. More recently blood samples have also been taken for murine typhus complement fixation tests; this will be reported on in a later paper. Only live-trapped rats were included in the survey. Rats found dead in or near the traps were examined only for signs of plague.

A total of 1066 rats were examined for ectoparasites; of these 773, or 72.6% were *Rattus norvegicus* and 293, or 27.4% were *R. rattus alexandrinus*. 57% of the Norway rats and 56.3% of the roof rats were females. The two species of rats have been found together in the port, in varying proportions, since 1933, although *R. rattus alexandrinus* is quite rare in the surrounding city of Haifa.

Fleas:

Xenopsylla cheopis, the Oriental rat flea, is by far the most common flea on both rat species. The seasonal distribution of *X. cheopis* is shown in Table I. This species reaches its peak in late August and early September with the high index of 32.0 for the month of August, 1954.

Petrie *et al* (1924) observed that the most favorable temperature for the development of *X. cheopis* was between 20° and 25° C. In late May 1954, the average weekly temperature, which had previously been below 20° C, rose to 24° along

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TABLE I — *Monthly indices, and percentage of rodents infested for all fleas and for* Xenopsylla cheopis

Month	Rodent Host Species	Rodent Host Number	All Fleas Number	All Fleas % Infested Rodents	*Xenopsylla cheopis* Number	*Xenopsylla cheopis* Index	*Xenopsylla cheopis* % Infested Rodents
			1954				
January	*R. norvegicus*	15	22	73.3	11	0.73	46.6
	R.rattus	14	3	14.2	0	0	0
February	*R.norvegicus*	18	9	27.2	1	0.05	5.5
	R.rattus	1	0	0	0	0	0
March	*R.norvegicus*	33	31	48.4	7	0.21	18.1
	R.rattus	14	3	14.2	0	0	0
April	*R.norvegicus*	33	79	81.8	34	1.03	54.5
	R.rattus	11	0	0	0	0	0
May	*R.norvegicus*	48	126	75.0	92	1.91	56.2
	R.rattus	8	12	50.0	8	1.0	50.0
June	*R.norvegicus*	48	423	91.6	391	8.14	89.5
	R.rattus	13	42	69.2	40	3.07	69.2
July	*R.norvegicus*	16	421	100.0	395	24.68	100.0
	R.rattus	10	137	100.0	134	13.40	100.0
August	*R.norvegicus*	42	1375	100.0	1361	32.40	100.0
	R.rattus	10	311	100.0	308	30.80	100.0
September	*R.norvegicus*	30	764	100.0	691	23.03	96.0
	R.rattus	50	1196	94.0	1154	23.08	94.0
October	*R.norvegicus*	20	366	95.0	265	13.25	95.0
	R.rattus	12	97	91.6	96	8.00	91.6
November	*R.norvegicus*	40	430	100.0	398	9.95	100.0
	R.rattus	9	43	88.8	33	3.55	88.8
December	R.norvegicus	36	232	88.8	213	5.91	86.1
	R.rattus	2	7	100.0	6	3.00	100.0
			1955				
January	*R.norvegicus*	12	18	50.0	15	1.25	33.0
	R.rattus	5	1	20.0	0	0	0
February	*R.norvegicus*	40	215	80.0	74	1.85	60.0
	R.rattus	25	180	84.0	37	1.48	36.0
March	*R.norvegicus*	45	561	97.7	464	10.31	86.6
	R.rattus	23	223	95.6	61	2.65	78.2
April	*R.norvegicus*	64	430	90.6	375	5.85	87.5
	R.rattus	11	64	45.4	37	3.36	45.4
May	*R.norvegicus*	39	275	94.8	230	5.89	89.7
	R.rattus	13	40	76.9	27	2.07	76.9
June	*R.norvegicus*	85	1424	94.1	1184	13.92	87.0
	R.rattus	25	107	52.0	93	3.72	52.0
July	*R.norvegicus*	109	1453	96.3	1236	11.34	94.5
	R.rattus	37	307	81.3	255	6.89	78.3

with a corresponding rise in the relative humidity to 71%. This was reflected 4 weeks later by a rapid increase in the *X. cheopis* index. The index begins to decline in late September.

The lowest percentage of infestation by *X. cheopis* of both the Norway and roof rat was found during the winter months. From July to November the rate of infestation, for all rats, was between 93% and 100% whereas in January it fell as low as 8%.

Unlike the results found by Davis (1951) in San Antonio, Texas, there is no very great difference between the index of *X. cheopis* on male and female rats of either species in Haifa port. The yearly index on male Norway rats is 9.3 and on females 9.8, while on roof rats it is 8.1 and 7.4 respectively.

During the winter and spring months, both the percentage of infestation and the *X. cheopis* index tend to be higher on the Norway rat than on the roof rat. It should be noted that generally, throughout the port, the nests of the Norway rat are found mostly in the ground or in other areas less exposed to radical changes of temperature than those of the roof rat.

Leptopsylla segnis is found mainly during the winter and early spring, being almost entirely absent during the hot summer months. As with *X. cheopis*, the Norway rat is generally more heavily infested although in no case are the numbers of this flea very high. The seasonal distribution of this species is shown in Table II.

Ctenocephalides felis was found too infrequently on the rats to warrant conclusions as to its seasonal distribution. A total of 46 *C. felis* was found on both species of rats during the course of the survey. No *C. canis* were found in this survey and it is generally scarce on rodents in Israel.

TABLE II — *Seasonal abundance of various ectoparasites*

Rodent Host			Ectoparasites							
			L.segnis		*E.murina*		*P.spinulosa*		*B.bacoti*	
Species	No.	Months	Index	%Inf.	Index	%Inf.	Index	%Inf.	Index	%Inf.
		1954								
R.norvegicus	66	I-III	0.51	24.2	0.90	7.5	0.98	33.3	4.16	62.1
R.rattus	29		0.20	13.7	0	0	0.44	24.1	2.86	55.1
R.norvegicus	129	IV-VI	0.55	20.3	0.28	17.0	1.66	34.8	12.76	67.4
R.rattus	32		0.03	3.1	0.12	6.2	0.31	20.6	6.65	40.6
R.norvegicus	98	VII-IX	0	0	1.00	32.6	0.13	5.1	7.24	44.8
R.rattus	70		0	0	0.72	25.7	0.35	11.4	6.30	52.8
R.norvegicus	96	X-XII	0.20	5.2	0.86	13.5	2.51	28.1	8.08	62.5
R.rattus	23		0.17	17.3	0.34	13.0	1.17	34.7	1.26	34.7
		1955								
R.norvegicus	96	I-III	1.90	34.3	0.43	17.7	2.00	44.7	4.31	64.5
R.rattus	53		5.40	64.1	0.30	13.2	1.96	39.6	7.01	58.4
R.norvegicus	188	IV-VI	0.33	19.3	1.30	23.4	1.59	36.7	7.29	72.8
R.rattus	49		0.51	12.2	0.59	10.2	0.79	22.4	6.04	48.9
R.norvegicus	109	VII	0.01	1.8	1.90	38.5	0.76	37.6	3.88	48.6
R.rattus	37		0	0	0.86	32.4	0.56	18.9	0.67	29.7

%inf. = percentage of rodent hosts infested with ectoparasite.

Despite the fact that earlier, unpublished survey records for Haifa port show *Nosopsylla fasciatus* as appearing very frequently, only 2 specimens were found in the survey, on 2 Norway rats trapped on March 28, 1954 in the same warehouse.

Echidnophaga murina is found throughout the year in comparatively small numbers with a slight rise in the late summer and fall. In all cases the seasonal index was higher for the Norway than for the roof rat. With the exception of fall, 1954, the percentage of infestation was also higher for the Norway rat. The data for this species are also summarized in Table II.

During the course of the above survey no other species of flea was taken on rats

trapped in the port. At a slightly later date, however, one *Stenoponia tripectinata* was found on a Norway rat trapped in a port warehouse.

Lice:

Two species of rat lice were recorded: *Polyplax spinulosa,* the spiny rat louse, and *Hoplopleura oenomydis.* The latter was found in small numbers, only rarely more than a few individuals being found on any one rat. The finding of *H. oenomydis* in this survey is a new record for the country. It has only previously been found in 1 instance on some laboratory rats in Jerusalem and on a dog kept in the same room as the rats. Aside from this, the species has not been recorded from any domestic or wild rodent and has been found only in the port area and its immediate environs. *H. oenomydis* is much more common on the Norway rat than on the roof rat; 28 infested Norway rats had 137 *H. oenomydis* whereas only 5 infested roof rats with 7 lice of this species were found. *Polyplax spinulosa* is more evenly distributed with a slightly higher yearly index for the Norway rat. There is only a slight fall-winter seasonal increase, (Table II).

Mites:

Three species of mites were commonly found on both species of rats: *Bdellonyssus bacoti* is found throughout the year, generally in quite large numbers, on both species of rats. Its seasonal distribution is shown in Table II. *Laelaps nuttali* is irregularly found throughout the year and *L. echidninus* appears in small numbers in the late spring and summer, being almost entirely absent during the winter and fall.

Ticks:

Only 1 *Rhipicephalus sanguineus,* the common dog tick of Israel, was taken from a Norway rat.

SUMMARY

Rats were trapped in the Haifa port area from January, 1954, to July, 1955, and examined for ectoparasites. Figures are given for the various ectoparasites found, as well as seasonal distribution for most species.

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FIVE NEW FLEAS OF THE GENUS *THRASSIS* JORDAN, 1929 (CERATOPHYLLIDAE: SIPHONAPTERA), A GENUS OF KNOWN IMPORTANCE IN PLAGUE TRANSMISSION

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Several members of the genus *Thrassis* serve to disseminate and maintain plague among certain groups of wild rodents in the western United States. Laboratory experiments (Eskey and Haas, 1940) show that several species have the ability to transmit plague. Macchiavello (1954) calculates the vector efficiency* of *T. pandorae* Jellison to be 1.10. Prince (1943) found that *T. bacchi bacchi* (Rothschild) had a ratio** of 0.10 transmissions to the 40 fleas used. Other species have been shown to have the following vector efficiencies: *T. francisi* (Fox) 0.09 and *T. acamantis* ssp., *T. stanfordi* Wagner and *T. arizonensis* ssp., all 0.04. (After Eskey and Haas, 1940). *T. petiolatus* (Baker) was laboratory-infected (Eskey and Haas, 1940) and naturally infected (Jellison, 1937). In addition to the above experimental findings, *T. bacchi pansus* (Jordan) *T. b. gladiolis* (Jordan) and *T. fotus* (Jordan) were reported by the San Francisco Field Station as being naturally infected. In surveys conducted over western United States by the San Francisco Field Station from 1936 to 1949 for determining the presence of plague in fleas, the genus *Thrassis* was more often in positive pooled inoculations than other genera. Most *Thrassis* collected and tested in the past have come from the larger rodents (Sciuridae). However, *Thrassis* of smaller rodents may play an important role in plague transfer. As an example *T. bacchi johnsoni* (Hubbard) has been shown to have a very high vector efficiency (Kartman and Prince, 1956). It occurs in great numbers on *Lagurus curtatus* ssp., the sagebrush vole, in eastern Washington and is probably the most important flea in maintaining plague in this rodent population.

Augustson (1944) discusses the significance of the distribution and host preference of the genus *Thrassis* to plague. Some members of the genus, like many common fleas, are quite catholic as to host preference and may disseminate plague to rodents other than their primary hosts. For example, *T. fotus* (Jordan) is taken almost as often from *Onychomys* sp. as it is from *Citellus* sp.

During the course of a systematic study of the genus *Thrassis* 5 species and subspecies heretofore unknown to science were encountered. The purpose of this publication is to describe these fleas and to discuss some of their relationships. The first 3 are parasitic on *Marmota* sp., and are closely related to *T. acamantis* ssp. and to *T. spenceri* Wagner. The last 2 parasitize groups of mammals in the subgenus Ammospermophillus and in the genus *Dipodomys* respectively.

Thrassis pristinus, new species

Thrasis (sic) *acamantis* (Rothschild) Philip, 1938, (*nec* Rothschild, 1905).
Thrassis acamantis (Rothschild) Jellison and Kohls, 1939; Jellison and Good, 1942 (*Partim*);

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* Vector efficiency is expressed as the average transmissions per flea having received an infectious blood meal by Wheeler and Douglas (1941).

** Vector efficiency was not calculable from the data presented. The ratio expressed closely approximates the actual vector efficiency.

Ewing and I. Fox, 1943 (*Partim*); Costa Lima and Hathaway, 1946 (*Partim*); Hubbard, 1947 (*Partim*); Holland, 1949, (*nec* Rothschild, 1905).

This species was first reported from Alaska as *T. acamantis* (Rothschild) by Philip, 1938, and subsequently by Jellison and Kohls, 1939, in their list of Alaskan fleas. Since these initial reports, it has been reported as *T. acamantis* from Alaska in bibliographies, monographs and text books dealing with fleas in general. Though definite morphological characters are present that may separate this flea from either *T. acamantis* or *spenceri*, it may be easily confused with either of these latter forms. Holland, 1949, suggested that differences might be found between Alaskan *Thrassis* and *T. spenceri* since the host *Marmota caligata* ssp. usually does not harbor *acamantis*.

Diagnosis:

This flea morphologically stands midway between *acamantis* and *spenceri*.

Male: Marked angle formed by the posterior border of tergum VIII in marmot fleas is more pronounced in *pristinus* than either *spenceri* ssp. or *acamantis* ssp. Apex of sternum VIII is less narrowed and sharp than in *acamantis* and more so than in *spenceri*. Finger narrower and longer than either that of *spenceri* or *acamantis;* distal end more pointed. Apex of crochet narrower and more pointed than in *spenceri*, less so than *acamantis*. *Female:* Upper part, posterior border sternum VII forms 90° angle to lower part; *spenceri* averages 102°; *acamantis* averages 115°.

General characteristics of significance—Chaetotaxy and morphological characters of head, thorax, legs and pregenital abdominal segments of both sexes similar to both *spenceri* and *acamantis* with the exception of the number of bristles of pedicel of antenna of male reaching at least half the length of the flagellum. These bristles number 5 (rarely 6) in *spenceri* and 7 (rarely 6) in *acamantis* and *pristinus*.

Modified segments—

Male: Tergum VIII, dorsal half: group of 8 to 12 large, long bristles variously arranged on each side near dorsum; ventral half: 8 to 10 long bristles on each side in upper group on posterior border and 17 to 25 short bristles on each side in lower group along ventral-posterior border. Sternum VIII narrow in apical half, gradually rounded ventrally, with 4 to 6 bristles along border, concave dorsally; apex sharply rounded; basal half broad. The VIII tergum and sternum best differentiate *pristinus* from *spenceri* and *acamantis*.

The marked angle formed by posterior border of tergum VIII in this group of fleas is more pronounced in *pristinus* than in either *spenceri* or *acamantis,* and apex of sternum VIII is less narrowed and sharp than in *acamantis* and more so than in *spenceri*. Finger does not exceed dorsal lobe of clasper in length; finger narrower and longer than either that of *spenceri* or *acamantis,* distal end more pointed; anterior border straight; 4 to 5 major bristles along posterior border. Acetabular bristles: 2 large, 0 to 1 small. Dorsal lobe of clasper more abruptly rounded at posterior dorsal angle as in *acamantis* (*spenceri* evenly rounded); posterior border sinuous to notched which latter condition is unlike either *acamantis* or *spenceri*. Manubrium slender, tapering as in other marmot fleas. Internal arms sternum IX bent at a sharper angle as in most *acamantis,* with ventral leg of angle being straighter as in most *spenceri*. Modified lateral bristles of the lower lobe similar to *spenceri* (long, straight, slender); apical bristle slightly shorter than lateral bristle. Ventral projection of the lateral lobe of the aedeagal apodeme slightly longer and sharper than that of *spenceri* but much less so than in *acamantis* which is extremely long. Apex of crochet more pointed and more narrow than in *spenceri,* less so than *acamantis*.

Female: Resembles both *acamantis* and *spenceri* in all recognizable morphological characteristics with the 2 following exceptions: Upper part, posterior border sternum VII forms 90° angle to lower part. This angle averages 102° in *spenceri* and 115° in *acamantis*. A ratio of the length divided by the greatest width of the anal stylet averages 3.2 among all specimens of *pristinus* examined (i.e. the stylet is 3.2 times as long as wide) whereas this ratio of stylet length to width averages 2.3 in *acamantis* and 2.4 in *spenceri*. While averages of the stylet ratio of the 6 type specimens differ from those of other species, unfortunately the individual measurements overlap those of extreme individuals of *acamantis* and *spenceri,* giving no reliable separation of *pristinus* females. Sternum VII has 7 to 8 bristles on each side, 2 to 4 small, thin bristles at ventral border. Arrangement of bristles on stylet and shape of the spermatheca appears as in either *acamantis* or *spenceri*. Accessory sclerotized cap (Traub, 1950) at tip of tail prominent in all specimens.

Length—Holotype male: 2.6 mm; average of 7 type males: 2.8 mm; range of type males: 2.4 mm to 3.3. Allotype female: 2.8 mm; average of 6 type females: 3.0 mm; range of type females: 2.7 mm to 3.7 mm.

Type locality: Seward, Alaska.

Type host: Marmota sp.

Type material: Type specimens are located in the Rocky Mountain Laboratory, Hamilton, Montana under file number A.P. 18168. The material was collected on July 15, 1937 by C. B. Philip of the Rocky Mountain Laboratory.

A male specimen from *Marmota* sp. found in Thompson Pass, Valdez Alaska, is also reported here. It was recovered July 13, 1948 by J. D. Gregson (Canadian Department of Agriculture). The specimen is deposited in the Canadian National Collection (File No. 3285).

It may be of significance that *T. pristinus* appears to fall between *T. acamantis* and *T. spenceri* in its morphological relationships. Holland (1949) states in the section on the relations of Nearctic and Palaearctic fleas, "The genera showing closest affinities to the Palaearctic forms occur across the north of Canada. Farther south and through the United States into Mexico and southward into South America, purely New World genera appear in increasing numbers and the more northern genera tend to become less common." No representative of *Thrassis* occurs in Asia, but its closest relatives are found there. The pattern of distribution of *Thrassis* in North America suggests a southward dispersal.

That *Thrassis* on marmots seem morphologically primitive when compared to other *Thrassis,* and that *Marmota* is generally conceded to be a primitive sciurid, suggests that marmot *Thrassis* represent the closest living form of a *Thrassis* prototype. If this be true, and the origin of *Thrassis* is northern, a prototype of *T. pristinus* would represent the ultimate origin of at least the *spenceri* and *acamantis* lines of marmot fleas and perhaps all *Thrassis.*

Thrassis spenceri vancouverensis, new subspecies

Thrassis acamantis (Rothschild). Spencer, 1936. (Note: Recorded from "Vancouver Island Marmot"; no locality given; according to Holland, 1949, record established on female); Holland, 1949, (*nec* Rothschild 1905).

Thrassis spenceri Wagner Holland, 1949, (*nec* Wagner 1936).

Diagnosis:

This flea is most closely related to the mainland form of *Thrassis spenceri* and is here named as a subspecies of *spenceri. Male:* Finger correspondingly shorter and wider than in *s. spenceri.* (3.7× longer than wide in *spenceri;* 2.8× longer than wide in *vancouverensis*). Apex of sternum VIII of *vancouverensis* more rounded than in *spenceri. Female:* Posterior border sternum VII slightly less angulate than in *spenceri.*

General characteristics of significance—Chaetotaxy and morphological characteristics of head, thorax, legs and pregenital abdominal segments similar to *T. s. spenceri.*

Modified segments—

Male: Tergum VIII, dorsal half: group of 10 to 13 large, long bristles variously arranged on each side near dorsum, 2 to 3 bristles placed laterally (6 to 12 bristles in *s. spenceri* with 0 to 3 placed laterally); ventral half: 5 to 6 long bristles on each side upper group on posterior border and 10 to 14 bristles on each side in lower group along ventral-posterior border. Bristles of this lower group are noticeably longer than those of *s. spenceri.* Sternum VIII gradually tapered to apex which is rounded; 3 to 7 bristles on each side along ventral border, the number varying from side to side on an individual specimen. The apex of sternum VIII of *s. vancouverensis* is more rounded than that of *s. spenceri.* Also apex usually projects farther beyond the most apical bristles in *s. spenceri* than in *s. vancouverensis* in which this bristle is placed on the apex. Finger of *s. vancouverensis* does not exceed clasper lobe in length, and it is correspondingly shorter and wider than in *s. spenceri.*

This fact was first mentioned by Holland in correspondence and is the most reliable and noticeable characteristic that may be used in separating these subspecies. Also anterior border of finger is more convex than in most specimens of *s. spenceri.* Measurements of length divided by width of finger give an average index number of 3.7 in *s. spenceri* and 2.8 in *s. vancouverensis,* there being no overlapping of extremes. Four to 5 major bristles along posterior border; acetabular bristles; 2 long, 1 to 2 short. Clasper lobe somewhat broader than in *s. spenceri;* manubrium as in *s. spenceri.* Internal arms, upper and lower lobes, and modified bristles of sternum IX as in *s. spenceri.* Entire aedeagus as in *s. spenceri.*

Female: Resembles *s. spenceri* in all recognizable morphological characteristics with exception of posterior border of sternum VII which is somewhat less angulate than *s. spenceri* and forms an angle which averages 120°. There is, however, some overlapping of extremes. Up to

13 bristles arranged in a row along each side of sternum VII. Ratio of stylet length to width is 2.3 which equals the average of *s. spenceri.* Spermatheca identical to that of *s. spenceri;* prominent accessory sclerotized cap at tip of tail.

Length—Holotype male: 2.8 mm.; average of 5 type males: 2.7 mm.; range of type males: 2.6 mm. to 2.8 mm.; allotype female: 3.0 mm.; average of 9 type females: 3.0 mm.; range of type females: 2.7 mm. to 3.3 mm.

Type Locality: Vancouver Island, British Columbia.

Type host: Marmota vancouverensis Swarth.

Collection date: August 30, 1943.

All type specimens can be found in the Canadian National Collection, Ottawa, Canada. The file number is 2371.

The females of the marmot fleas are extremely difficult or impossible to separate, which possibly shows close relationship among members of this group of the genus *Thrassis.* As stated by Holland, 1949, when discussing *T. spenceri,* "In general, males are necessary for identification." Male characteristics are sufficient base to use for the separation of species or subspecies as long as they indicate morphological differences of whole populations. The difficulty in identifying females in this group is that there are no known characteristics which man's observation may rely upon, and there is no method available at present which will separate *T. s. vancouverensis* females from other marmot fleas except by locality or accompaniment of males.

It was mentioned by Holland in 1949 that the specimens from Vancouver Island showed slight differences, but he stated that differences of these fleas from isolated alpine marmots may be within individual variation of mainland forms. In a personal communication, Holland mentioned the fact that *Marmota vancouverensis* belongs to the *caligata* group, but is considered specifically distinct, and is believed by some students to have survived the glaciation on a nunatak. With all due regard for caution, I believe that the biometric measurement of the finger may not eliminate the possibility of overlapping individual variation, but it definitely established the presence of a different range of variation in the Vancouver Island population from that of *s. spenceri* on the mainland.

Thrassis alpinus, new species

Diagnosis:

This species is closely allied to the mainland form of *spenceri. Male:* Crochet noticeably long, having straight dorsal and ventral borders through posterior half; apex not a sharp point as in *acamantis* but not as blunt as *spenceri.* Other characteristics of this species are similar to those of *spenceri* except that 18–26 bristles are located in the group of bristles along the posterior border of tergum VIII, while those of *spenceri* do not number above 16. *Female:* Posterior border sternum VII with sharply rounded angle of 110°.

General Characteristics—Chaetotaxy and morphological characteristics of head, thorax, legs, and pregenital abdominal segments similar to *T. spenceri* ssp. and *T. acamantis* ssp. in both sexes.

Modified segments—

Male: Tergum VIII, dorsal half: group of 6 to 12 large, long bristles, variously arranged on each side near dorsum; ventral half: 4 to 6 long bristles on each side in upper group on posterior border placed some distance from apex and 18 to 26 shorter bristles on each side in the lower group along the ventral posterior border, as in *T. acamantis.* (Those of *T. a. utahensis* Wagner often number above 30 and those of *spenceri* number 16 or fewer.) The remaining characteristics ally *alpinus* with *spenceri.* Sternum VIII differs but slightly from that of *spenceri;* ventral border little curved toward apex with dorsal border being parallel in apical portion; apex rounded or bluntly pointed; 5 to 6 bristles, each side with 0 to 2 smaller bristles anterior to these along ventral border. Finger not exceeding dorsal lobe of clasper; with 5 major bristles, the uppermost being rather small in some specimens. Dorsal lobe of clasper more abruptly rounded as in *pristinus* (less so than *acamantis*); with 3 or 4 minute bristles. Acetabular bristles: 2 large, 2 to 3 short bristles placed below. Manubrium narrow

apically, rounded to bluntly pointed. Internal arms sternum IX slender, angulate with ventral leg of angle usually straighter as in *spenceri* and *pristinus;* not curved as in *acamantis* (curved in one specimen); lateral modified bristles on lower lobe long and narrow as in *spenceri.* Ventral projection of lateral lobe of aedeagal apodeme about as prominent as in *spenceri* but is much broader and more blunt than in any other marmot flea (except *T. stanfordi* which is extreme). Neck of aedeagus thicker than *acamantis acamantis* and *spenceri,* being similar to that of *acamantis howelli* (Jordan). Crochet long, having straight dorsal and ventral borders through posterior half; apex not a sharp point as in *acamantis* ssp. but not as blunt as *spenceri.*

Female: (single specimen) Posterior border sternum VII with rather sharply rounded angle of 110°; upper part curved high dorsally; 8 bristles on each side, 2 of which are placed dorso-anteriorly; 5 small bristles at ventral border. Stylet length to width = 2.45. Stylet bristles: 1 long dorsal, 1 long ventral, no lateral, and 1 long apical. Spermatheca typical of all marmot fleas; accessory sclerotized cap at tip of tail prominent. While this description may appear accurate, it refers only to the single female specimen available which may or may not represent an average of possible variations.

Length—Holotype male: 3.40 mm.; average of all males: 2.76 mm.; range of males: 2.45 mm. to 3.40 mm.; female: 3.47 mm.

Type locality: Mt. Evans, Clear Creek Co., Colorado; 13,500 ft. in elevation, above timber.

Type host: Marmota sp.

Type material: holotype male, paratype male; No. 271:49 on deposit at San Francisco Field Station, San Francisco, California. Collected July 14, 1949 by Dean H. Ecke, P.H.S.

Other material: 7 males, 1 female from *Marmota* sp.; Boulder County, Colorado; June, 1940. On deposit at Rocky Mountain Laboratory, Hamilton, Montana (National Institute of Health); No. A.P.16786.

T. alpinus appears most closely related to *T. spenceri,* and, with study, additional material may prove the former to be a subspecies of the latter. The preferred host of *spenceri* is *Marmota caligata* ssp.; in fact, the preference is so marked that specimens of *spenceri* may be fairly reliably separated from *acamantis* (on *M. flaviventer*) simply on the basis of the host on which they are found. Unfortunately, the identification of the host from which the type specimens of *T. alpinus* were taken is uncertain and no study skin was prepared. The marmot from which the holotype was taken was keyed to *caligata,* but later the identification was changed to *M. f. luteola* Howell since *caligata* was not known from Colorado. Therefore, it is necessary to omit the species name of the host of the holotype flea, in view of uncertainty of the identity of *M. f. luteola* and the high degree of preference of this group of fleas for *M. caligata.* In spite of the fact that *caligata* has not yet been reported (Howell, 1915 and Warren, 1910), I am not convinced that *M. caligata* (*nivaria* Howell or ssp.?) might not someday be discovered at 13,000 ft. elevation on Mount Evans, Colorado, or some nearby area.

Thrassis bacchi consimilis, new subspecies

Thrassis bacchi new ssp. Stark, 1955.

This subspecies fits into the pattern of distribution of the *Thrassis bacchi* complex.

Diagnosis:

This subspecies is most closely related to *T. b. pansus* (Jordan) and *T. b. setosis* Prince. *Male:* Lower lobe sternum IX with modified lateral bristles at or very close to level of apical bristle so that all bristles are arranged parllael or nearly so; whereas in both *pansus and setosis* one of these bristles is placed at a lower level than the other lateral bristle and the apical bristle. Apex of manubrium is often expanded or even widely flared. It is rarely expanded in *pansus.* The flea further differs from *setosis* which has numerous large bristles (8–9) along the posterior border tergum VIII (not more than 4 in *consimilis*). *Female:* Not reliably separated (as is typical of all females of this complex).

General characteristics—Chaetotaxy and morphological characteristics of head, thorax, legs, and pregenital abdominal segments of both sexes similar to those of *T. pansus* and *T. setosis.*

Modified segments—

Male: Tergum VIII extends over parts of other modified segments. Distinct group of 3 or 4 long bristles and 2 or 3 short bristles in dorsal part. Three rarely 4, long bristles near posterior

border in ventral part, resembling *pansus* most closely. Sternum VIII broadly rounded as in pansus, not highly modified; a distinct supraventral sclerotized line present; a group of 4 to 5 short bristles is above and below this line on each side. Finger exceeds or equals dorsal lobe of clasper in length and ends bluntly at apex. Four to 6 major bristles along posterior border. Two shorter bristles in ventral half and 1 or 2 very long bristles in dorsal half. Anterior margin has a prominent angle in middle, the upper leg of angle slanting posteriorly to apex of finger. Dorsal process of clasper narrowly rounded with 2 or 3 minute bristles. Manubrium tapers from base to three-quarters length of apex and then is expanded either gradually to a square end or abruptly to a widely flared end in the majority of specimens. Dorsal angle of apex of manubrium more widely flared than ventral. In *pansus*, apex of manubrium is not expanded or it may have a slight flare; dorsal angle usually not widely flared. Internal arms of sternum IX slender with a 130° angle located about three-quarters toward apical end of arm. Lower lobe sternum IX with modified lateral bristles at or very close to level of apical bristle; all bristles arranged parallel or nearly so. One to 3 small thin bristles below these. In *pansus* and *setosis* one lateral bristle (one nearest border) is at lower level. Upper lobe sternum IX wide, membranous, with many thin bristles. Aedeagal apodeme thin, similar to that of *pansus* and *setosis*. Crochet thinner than in *pansus* and *setosis*, especially through the mid portion. Apex not as strongly extended posteriorly into a thin prominent angle as in *pansus*.

Female: Not as reliably separated from *pansus* as male. Posterior border sternum VII undulant, slanting anteriorly at a curved angle; *pansus* usually not as angulate. Four long and 2 short bristles arranged in row; 2 small bristles placed anterior to row. Stylet length to width ratio = 2.07 *consimilis* and 1.96 *pansus*. Bristle arrangement of stylet in 9 out of 10 *consimilis* specimens is: 1 long dorsal, 1 long ventral, 1 medium lateral, and 1 long apical. In *pansus* 2 out of 3 specimens studied are as above but 1 out of 3 have the following bristle arrangement on stylet: 2 long dorsal, 2 long ventral, 1 medium lateral and 1 long apical. Spermatheca quite like *pansus* except head not as globular and accessory sclerotized cap at end of tail often lacking as is typical in this group of *Thrassis*.*

Length—Holotype male: 2.15 mm.; average of type males: 1.99 mm.; range of type males: 1.6 to 2.3 mm.; allotype female: 2.6 mm.; average of type females: 2.61 mm.; range of type females: 2.4 to 3.1 mm.

Type locality: 23 miles southwest of Holbrook, Navajo Co., New Mexico; 5900 ft. elevation; ecological association: cedar, pine, juniper, grass, sandy soil, scattered rock formation on slope.

Type host: Citellus spilosoma cryptospilotus (Merriam)

Type material: No. B1876: 39 on deposit at San Francisco Field Station, San Francisco, California.

Collection date: Two males and 5 females were collected May 10, 1939.

Localities of other material: Arizona; Conconino Co.; 16 to 20 mi. W. Winslow; 4800 to 5000 ft.; April 12, and 28, 1939, August 22, 1939; 1 male, 8 females from *Citellus leucurus cinnamomeus* (Merriam). Navajo Co.; 2 mi. E. Holbrook; 5000 ft., sage; April 29, 1940; 1 male, 2 females from *Onychomys leucogaster* ssp.; 5 mi. S. Holbrook; 4800 ft., juniper; August 23, 1949; 4 males, 3 females from *C. l. cinnamomeus* (Merriam); 4 mi. N. W. Snowflake; 6000 ft., cedar, canyon, rocks; May 11, 1939; 1 male, 1 female from *C. l. cinnamomeus* (Merriam); 10 mi. N. E. Winslow; 5000 ft., desert, ravine, sand, sage; May 4, 1939; 1 female from *C. l. cinnamomeus* (Merriam); also 2 males, 3 females from *O. leucogaster* ssp. Colorado; Dolores Co.; 2 mi. S. Northdale; 7000 ft., grass, sage; August 4, 1939; 8 males, 7 females from *Citellus leucurus* ssp.; 6 mi. S.W. Sane Creek; 6800 ft., grass desert; August 1, 1939; 1 male, 3 females from *Citellus spilosoma* ssp. New Mexico; San Juan Co.; 3 mi. E. Blanco; 6000 ft., juniper; May 21, 1949; 1 male, 1 female from *C. l. cinnamomeus* (Merriam). Utah; San Juan Co.; 2 mi. S. Blanding; 6000 ft.; April 19, 1937; 1 female from *Cynomys gunnisoni zuniensis* Hollister; 15 mi. N. E. Bluff; 4700 ft., grass, sage, cedars; August 21, 1939; 2 females; *Citellus leucurus* ssp.; 2 mi. N. Bluff; 4500 ft., desert; August 24, 1939; 1 male from *Citellus variegatus grammurus* (Say); 1 mi. N. Mexican Hat; 4200 ft., desert, grass, sage, cacti; August 26, 1939; 2 males, 1 female from *Citellus leucurus* ssp. All above material was collected by members of the field units of the San Francisco Field Station.

Some of the above specimens have been sent to the following museums: U. S. National Museum, Washington, D. C., British Museum of Natural History, Tring, England; Canadian National Museum, Ottawa, Canada; Communicable Disease Center, U. S. Public Health Service, Atlanta, Georgia; University of California Museum, Berkeley, California.

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* The presence or prominence of the accessory sclerotized cap (Traub, 1950) is of no great taxonomic significance. The clearing process may cause this structure to disappear, though it is more often found in some groups of fleas than others and is an aid in recognition. A description of this structure is given by C. Fox, 1914.

Thrassis arcuatus, new species

Diagnosis:

Most closely allied to the *aridis, campestris, hoffmani* group. This group parasitizes *Dipodomys* sp., and may be separated from other *Thrassis* by the presence of 4 to 6 darkly pigmented spinelets on the posterior border of the metanotum. *Male:* Posterior border of finger curved in narrower arc than in *aridis, campestris* or *hoffmani.* Apex of sternum VIII narrower than any of the preceding fleas. *Female:* Posterior border sternum VII without lobe or sinus as is found in *aridis, campestris* and *hoffmani.*

General Characteristics—Chaetotaxy and morphological characteristics of head, thorax, legs, and pregenital abdominal segments of male and female similar to *T. aridis* Prince 1944, *T. campestris* Prince 1944 and *Thrassis hoffmani* (Hubbard 1949). My reference to *Thrassis hoffmani,* placed in the genus *Thrassoides* Hubbard 1947 by Hubbard in 1949, is intended only to compare relationships of related species and not to revise the status of the genus *Thrassoides.* These unique members of *Thrassis* occurring on *Dipodomys* are separated from most other *Thrassis* by the presence of 4 to 6 darkly pigmented spinelets on the posterior border of metanotum and by some features of the modified segments (e.g. balloon-like aedeagal apodeme).

Modified segments—

Male: Tergum VIII similar to *aridis, campestris and hoffmani,* barely covering dorsal half of modified segments, exposing ventral half. Dorsal group of 4 to 7 medium bristles and ventral group of 2 to 3 long bristles on tergum VIII. Sternum VIII not highly modified, fairly similar in appearance to other abdominal sterna. Four to 6 long bristles grouped at apex of posterior margin. Usually no small bristles located anterior to the apical bristles as in *campestris,* or not more than 2 small bristles as against 4 or more in *campestris.* Apex sternum VIII narrower than *aridis, campestris,* or *hoffmani.* Posterior border of finger curved, more so than in any of the preceding fleas, hence the name *arcuatus;* the greatest curvature at the middle of the posterior border; apex blunt. The posterior border of *aridis* and others is quite vertically straight. Four to 5 major bristles along the posterior border, the 2 or 3 lowermost bristles being stouter than the upper two. (One paratype has 3 bristles on left side, 2 on right.) Dorsal lobe of clasper shaped as in preceding species, armed with 3 or 4 minute bristles. Manubrium thin, gradually tapered from base to apex as in *aridis,* but apex not curved dorsally as much as in *aridis.* Inner arms sternum IX similar to *aridis, campestris* and *hoffmani,* those of *campestris* being slightly thicker throughout than those of the other three fleas. Lower lobe sternum IX not broadly curved as in preceding fleas; 3 small thin bristles present on each side plus 1 apical bristle as in *aridis;* upper membranous lobe short as in *aridis,* quite unlike *campestris;* anterior border straight while in *hoffmani, aridis* and *campestris* it is convex. The aedeagus of all 4 *Thrassis* occurring on *Dipodomys* varies little. The submedian mesal lobe of the apodemal strut is shorter in *arcuatus* than in preceding fleas. The crochet is shorter apically and the dorsal border is straight, not concave.

Female: Posterior border sternum VII varies from straight to markedly undulant, but neither lobe nor sinus is present in any of the specimens examined as it is in *aridis, campestris* and *hoffmani.* Posterior row of 7 to 8 large bristles on each side sternum VII; 2 to 4 anterior bristles arranged in either a group or row. Seventeen to 18 bristles on sternum VIII small and thin, grouped more closely together when compared to *aridis* and *campestris.* Bristles of sternum VIII of *hoffmani* midway between condition in *arcuatus* and in *aridis.* Stylet of allotype slightly shorter and broader than in preceding fleas: length to width ratio is 2.30 (average of all type specimens: 2.58). All 3 preceding fleas have higher average index numbers of stylet length to width ratio. Bristle arrangement of stylet for majority of specimens as follows: 1 medium and 1 minute dorsal, 1 medium and 1 long ventral, 1 medium lateral, and 1 long apical. Spermatheca quite distinct, resembling that of *hoffmani,* but head not as large and it is quite globular. The most significant character of spermatheca is the more heavy chitinous layer around the head extending over basal third of tail appearing as a sheath or ring. Tail long with an abrupt angle of 90° to 100°. Apical end of tail with definite accessory sclerotized cap.

Length—Holotype male: 1.90 mm.; average of type males: 2.01 mm.; range of type males: 1.80 to 2.20 mm.; allotype female: 2.20 mm.; average of type females: 2.51 mm.; range of type females: 2.1 mm. to 3.0 mm.

Type locality: Hunter Liggett Military Reservation, 5 mi. North of Jolon and 13 miles southwest of King City, Monterey County, California; 900 ft. elevation; ecological association: river bottom, grass, brush, willows.

Type host: Dipodomys heermanni jolonensis (Grinnell)

Type material: Holotype male, allotype female No. 51:190; paratypes: No. 51:190, 51:191, 51:192 on deposit at San Francisco Field Station, San Francisco, California. All above material was collected by members of the field units of the San Francisco Field Station.

Collection date: Eleven males and 12 females collected February 26, 1947.

Some of the above paratypes have been sent to the following museums: U. S. National Museum, Washington, D. C., British Museum of Natural History, Tring, England; Canadian National Museum, Ottawa, Canada; Communicable Disease Center, U. S. Public Health Service, Atlanta, Georgia; University of California, Berkeley, California.

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EXPLANATION OF FIGURES

*Abbreviations**

A. B.	Acetabular bristles.
AE. A.	Aedeagal apodeme.
A. I. T.	Armature of inner tube.
A. L. L.	Accessory lateral lobe of aedeagus or extension of lateral plate of aedeagal apodeme.
A. S. C.	Accessory sclerotized cap at apical end of tail of spermatheca.
B.	Angle formed at posterior border of Tergum VIII.
B. C.	Bursa copulatrix.
CR.	Crochet.
C. S.	Crescent sclerite.
D. S.	Ductus seminalis (to spermatheca).
D. S. C.	Ductus seminalis communis.
D. VIII	Dorsal half, posterior part of Tergum VIII.
F.	Finger of clasper lobe.
G. L.	Lower group of bristles on ventral half of posterior border of Tergum VIII.
G. U.	Upper group of bristles on ventral half of posterior border of Tergum VIII.
L. L.	Lateral lobe of aedeagus.
M.	Manubrium.
M. D. L.	Median dorsal lobe of aedeagus.
N.	Neck of aedeagus.
P.	Dorsal or immovable process of clasper lobe.
P. A.	Proximal or internal arm of sternum IX.
P. S.	Proximal spur of aedeagus.
Sperm.	Spermatheca or receptaculum seminis.
S.	Sternum (of abdominal segment).
T.	Tergum.
T. AP. IX	Ventral margin of apodeme of tergum IX.
U. L.	Upper lobe of distal arm of sternum IX.
V. L.	Lower lobe of distal arm of sternum IX.
V. VIII	Ventral half, posterior border of Tergum VIII.

* The terms applied to the reproductive organs in these drawings are essentially the same as those applied by Traub, 1950, in his comparative studies of the aedeagus. Since this paper is descriptive and no comparative morphology is intended, a limited number of terms are used to serve as landmarks in the identification of parts drawn.

List of Illustrations of type specimens

1. *Thrassis pristinus* ♀ Sternum VII and spermatheca
2. " " ♂ Tergum VIII
3. " " ♂ Sternum VIII
4. " " ♂ Sternum IX
5. " " ♂ Tergum IX, clasper, manubrium
6. " " ♂ Aedeagus
7. *T. spenceri vancouverensis* ♀ Sternum VII and spermatheca
8. " " " ♂ Tergum VIII
9. " " " ♂ Sternum VIII
10. " " " ♂ Sternum IX
11. " " " ♂ Tergum IX, clasper, manubrium
12. " " " ♂ Aedeagus
13. " " *spenceri* ♂ Sternum VIII
14. " " " ♂ clasper
15. *T. alpinus* ♂ Paratype, clasper
16. " " ♂ " cröchet of aedeagus
17. " " ♀ Sternum VII (not a type specimen)
18. " " ♂ Tergum VIII
19. " " ♂ Sternum VIII
20. " " ♂ Sternum IX
21. " " ♂ Tergum IX, clasper, manubrium
22. " " ♂ Aedeagus
23. *T. bacchi consimilis* ♀ Sternum VII and spermatheca
24. " " " ♂ Tergum VIII
25. " " " ♂ Sternum VIII
26. " " " ♂ Sternum IX
27. " " " ♂ Tergum IX, clasper, manubrium
28. " " " ♂ Aedeagus
29. *T. arcuatus* ♀ Sternum VII and spermatheca
30. " " ♂ Tergum VIII
31. " " ♂ Sternum VIII
32. " " ♂ Sternum IX
33. " " ♂ Tergum IX, clasper, manubrium
34. " " ♂ Aedeagus
35. " " ♂ Paratype, tergum IX, clasper, manubrium
36. " " ♂ Aedeagus

PLATE I

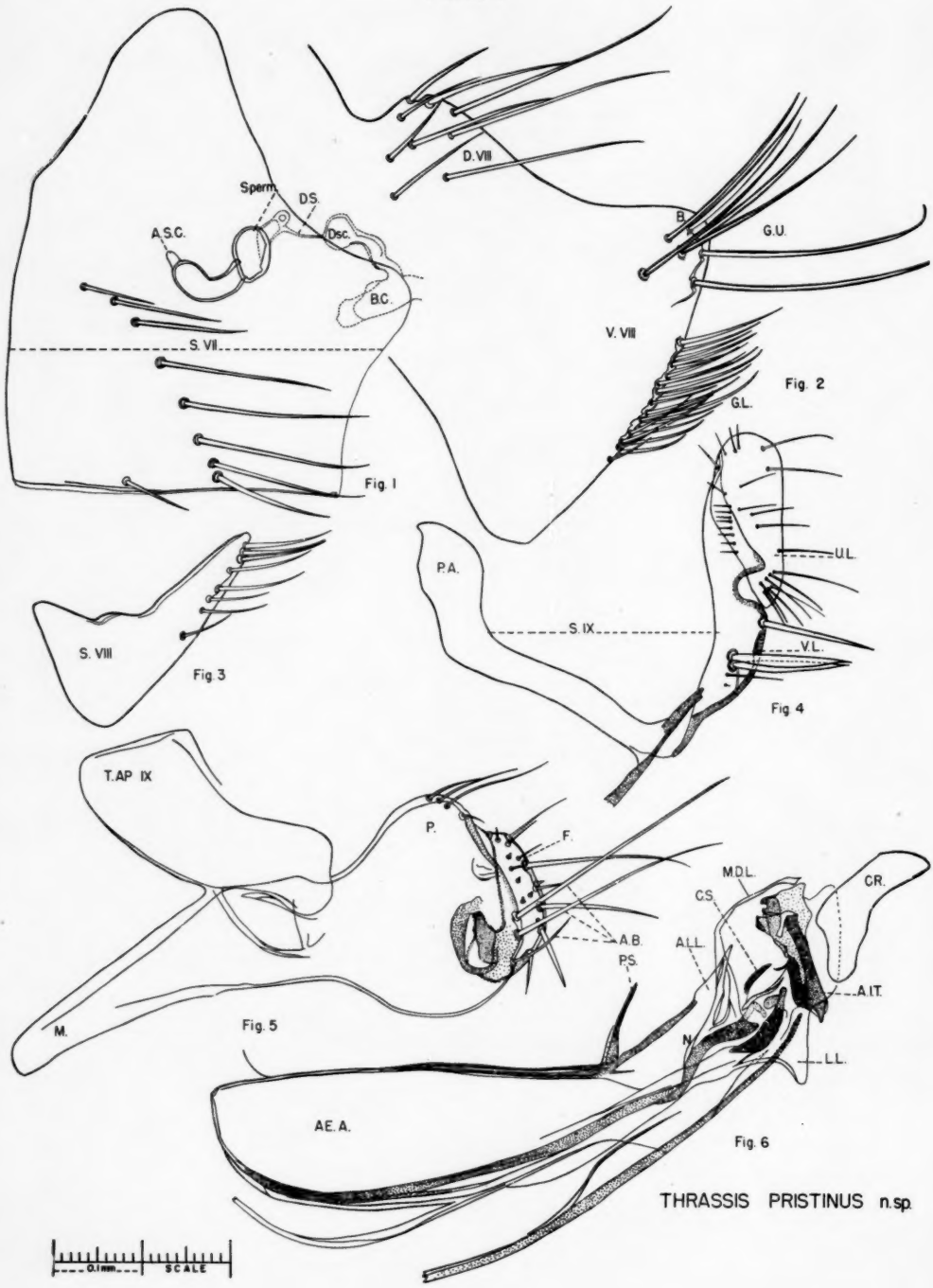
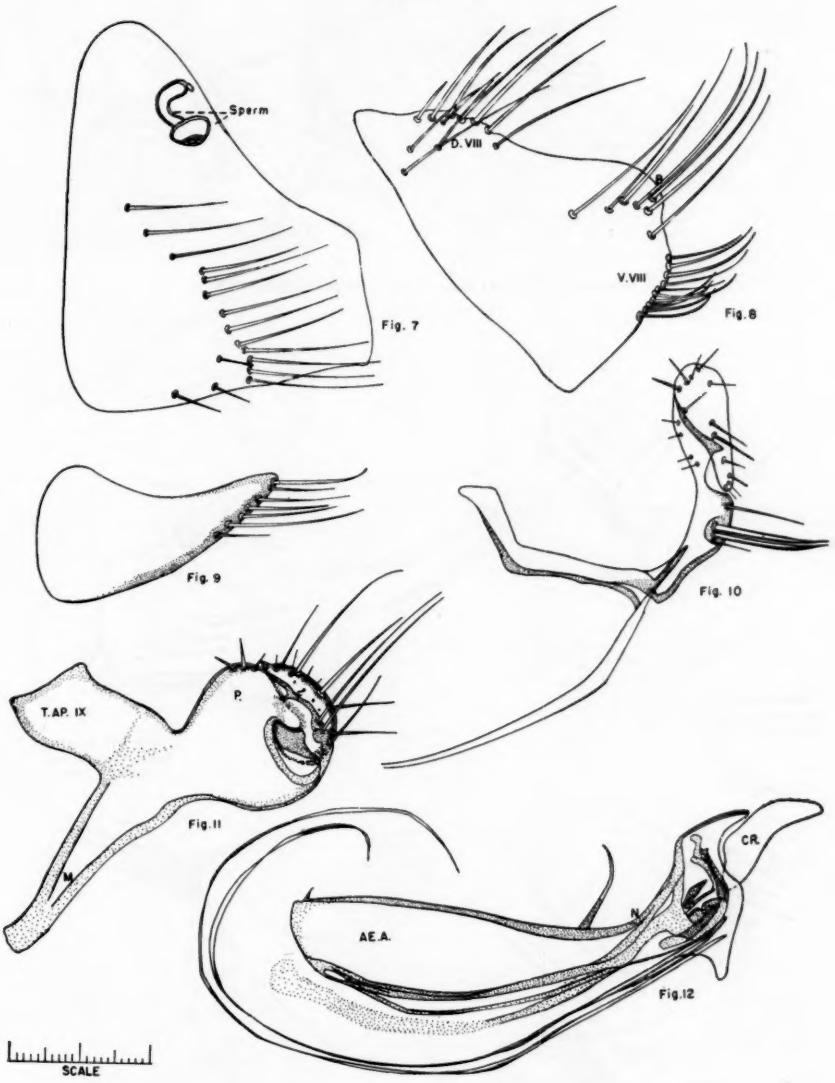

Sperm.
DS.
A.S.C.
Dsc.
B.C.
S. VII
Fig. 1
D.VIII
B.
G.U.
V. VIII
Fig. 2
G.L.
U.L.
V.L.
P.A.
S. IX
Fig. 4
S. VIII
Fig. 3
T.AP IX
P.
F.
A.B.
P.S.
M.
Fig. 5
M.D.L.
C.S.
CR.
A.L.L.
A.I.T.
N.
L.L.
AE. A.
Fig. 6
THRASSIS PRISTINUS n.sp.
0.1mm.
SCALE

PLATE II



THRASSIS SPENCERI VANCOUVERENSIS n. ssp.

PLATE III

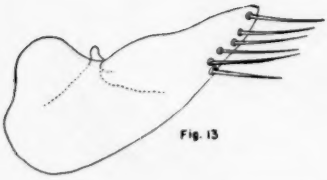


Fig. 13

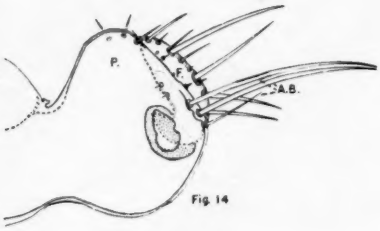


Fig. 14

THRASSIS S. SPENCERI Wagner Near type locality (Dunn Peak, B.C.)

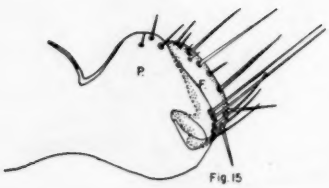


Fig. 15

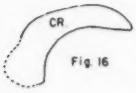


Fig. 16

Paratype male from
Mt. Evans – type local.
(T. ALPINUS n. sp.)

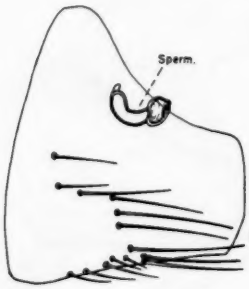


Fig. 17

Female from Marmota sp.
Boulder Co., Colorado
(T. ALPINUS n. sp.)

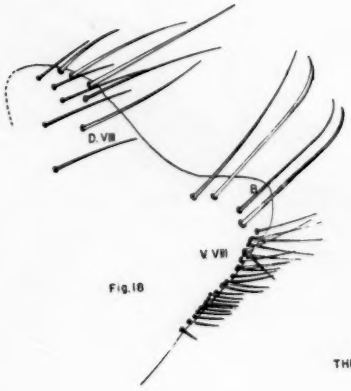


Fig. 18

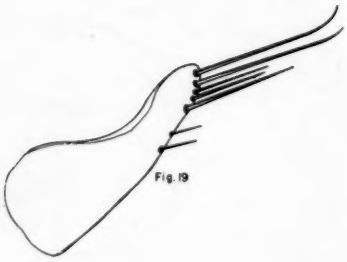
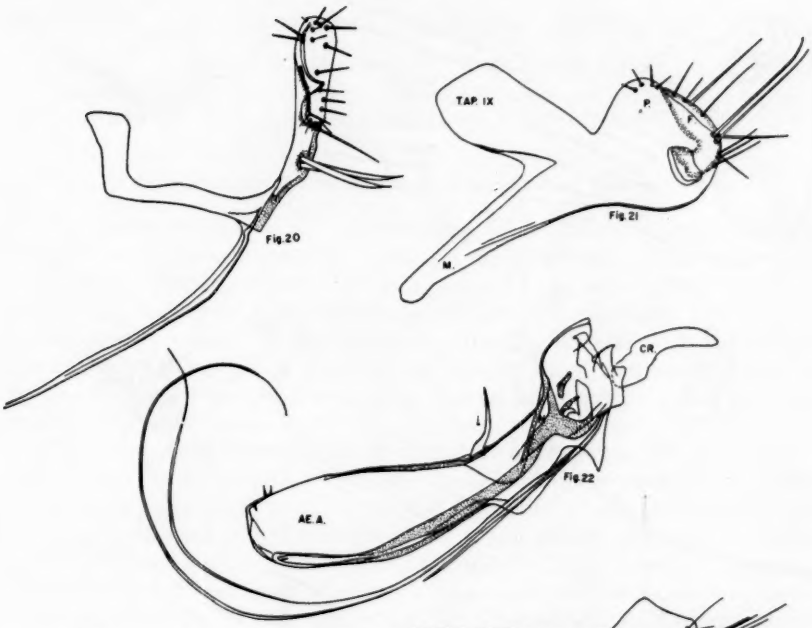


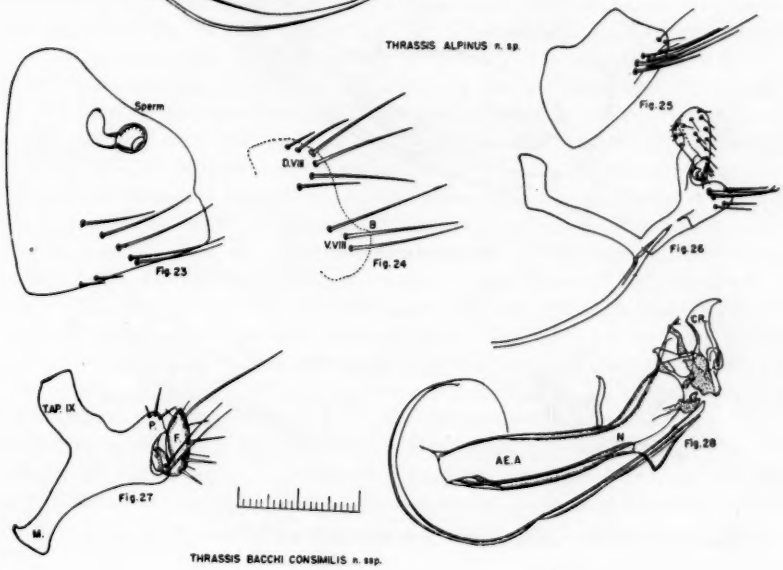
Fig. 19

THRASSIS ALPINUS n. sp.

PLATE IV

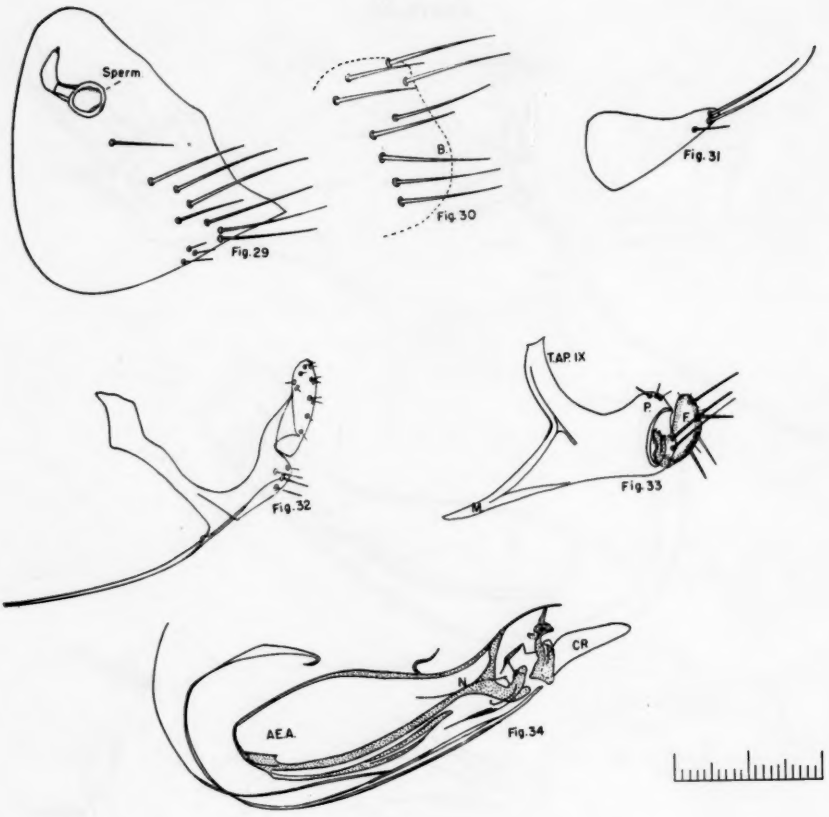


THRASSIS ALPINUS n. sp.

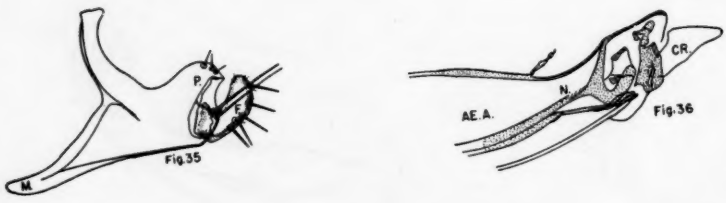


THRASSIS BACCHI CONSIMILIS n. ssp.

PLATE V



THRASSIS ARCUATUS n. sp.



Paratypes of T. arcuatus showing variation in the finger and crochet

NOTES ON SOME ANOPLURA FROM NEW MEXICO AND MEXICO

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Observations on 11 species of Anoplura from 22 species of rodents are recorded to supplement existing knowledge of biology and distribution. The most extensive collection records are from Santa Fe County, New Mexico. Other New Mexico counties represented in the records include Bernalillo, Sandoval, Otero, Luna, Socorro, and Torrance. A few records are from the northern part of the State of Chihuahua, Mexico. The notes include information on seasonal abundance of females, males, and nymphs from specific host animals together with a few brief morphological observations by the junior author.

The records from Santa Fe County were made in connection with a Communicable Disease Center field study to observe the biological relationships of mammals, ectoparasites, and sylvatic plague. A general description of the study has been prepared by Holdenried and Morlan (in press). The present report gives records and information related to the more than 3,000 sucking lice, representing 11 species, collected from 8,784 mammals examined or re-examined for ectoparasites in Santa Fe County from July, 1951, to February, 1954. No effort was made to collect more than a sample of lice from heavily infested animals. While consistent efforts were made to obtain ectoparasites from all live and dead animals examined, light infestations of lice on live animals were probably missed on many occasions. Comprehensive seasonal data were obtained for a two-year period, October, 1951, through September, 1953, by intensive weekly collections at the Santa Fe Municipal Airport.

Records of Anoplura from Bernalillo and other New Mexico Counties (except Santa Fe), and records from Chihuahua, Mexico (south of El Paso, Texas, and Columbus, New Mexico), are based on collections made by the junior author during 1948 and 1949. During this period, 261 rodents were examined for ectoparasites. Of these, 55 rodents were taken in Mexico and 151 were obtained from Bernalillo County, New Mexico, with smaller numbers from each of the other New Mexico Counties. A total of 606 lice, representing eight species, were collected and identified to species. Of these, 507 lice, including representatives of all eight species, occur in collections from Bernalillo County. Field collecting in central New Mexico was done by the junior author with the aid of a faculty research grant from the University of New Mexico, and collecting along the Mexican border and in the State of Chihuahua was supported by a grant from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

The species of Anoplura in the following account are arranged in the order given by Ferris (1951). All species are included in the family Hoplopleuridae.

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Enderleinellus suturalis (Osborn)

Our specimens agree in detail with the description given by Ferris (1920). This species has been collected in many localities from Alaska to Mexico in the western part of North America and is known from China and Siberia (Ferris, 1951). The usual hosts are species of ground squirrels (*Citellus*), but there are a few records of specimens taken from prairie dogs (*Cynomys*).

RECORDS: BERNALLILO COUNTY: Four females from one of 12 specimens of *Citellus spilosoma marginatus* Bailey taken from the University of New Mexico golf course; SANTA FE COUNTY: one female and five males taken in May from one of 326 specimens of *C. s. marginatus;* CHIHUAHUA: 12 females, seven males and four nymphs from a single specimen of *C. tereticaudus* (Baird) taken in June along the El Paso–Chihuahua City highway about 55 kilometers south of Ciudad Juarez.

Hoplopleura acanthopus (Burmeister)

This species is typically an ectoparasite of various kinds of voles (*Microtus*), but there are a few records of infestations from rodents of other genera (Ferris, 1951). Our only available specimens of *Microtus* were obtained in Santa Fe County.

RECORD: SANTA FE COUNTY: One male and one nymph taken in October from one of 41 *Microtus longicaudus mordax* (Merriam).

Hoplopleura arboricola Kellogg and Ferris

Western chipmunks (*Eutamias*) are the usual hosts for this sucking louse (Ferris, 1951).

RECORDS: BERNALILLO COUNTY: Three females and three males from four of 15 *Eutamias quadrivittatus quadrivittatus* (Say) taken in March and May, Sandia Mts., east of Albuquerque; SANTA FE COUNTY: 38 females, three males, and two nymphs collected during April, May, August, and September from three of 88 *E. q. quadrivittatus,* and from one of 19 *E. minimus operarius* Merriam.

Hoplopleura hesperomydis (Osborn)

Our specimens agree well with the description given by Ferris (1921), except that in a few specimens the paratergal plates of the mid-part of the abdomen appear relatively wider than shown by Ferris (1921, Fig. 39-A) and by Pratt and Lane (1951, Fig. 7) and the emargination is often a little wider and somewhat more shallow. Also we have observed in a few specimens that the larger of the two setae in the emargination of the fifth and sixth paratergal plates is longer than shown in the figures mentioned above, this seta sometimes reaching virtually to the posterior margin of the plate. *Hoplopleura hesperomydis* is typically an ectoparasite of deer mice (*Peromyscus*) and grasshopper mice (*Onychomys*). The species has been reported from North and South America and from Turkestan and China (Ferris, 1951). While we have taken large numbers of adult *H. hesperomydis* in our central and northern New Mexico collections during all months of the year except February, we have found only eight nymphs. Four of these were taken from *Onychomys leucogaster pallescens* Merriam in March in Santa Fe County and four were obtained in April from a specimen of the same species in Bernalillo County.

RECORDS: BERNALILLO COUNTY: 64 females and 21 males from 25 of 65 *Peromyscus maniculatus* (Wagner); 24 females and 24 males from five of 25 *P. truei truei* (Shufeldt); one female from one of three *P. boylei rowleyi* (Allen); five females, two males, and four nymphs from two of five *Onchomys leucogaster* (Weid); OTERO COUNTY: two females from one specimen of *O. leucogaster* taken seven miles south of Alamogordo; SANDOVAL COUNTY: 14 females and eight males from six of eight *P. maniculatus;* SANTA FE COUNTY: eight females and two males from two of 41 *Microtus longicaudus mordax* (Merriam); three females, two males, and four

nymphs from two of 405 *Onychomys leucogaster pallescens;* two females from two of 285 *Peromyscus leucopus tornillo* Mearns; 16 females and two males from nine of 445 *P. maniculatus rufinus* (Merriam); one female from one of 21 *P. nasutus nasutus* (Allen); 11 females and six males from six of 662 *P. truei truei* (Shufeldt); CHIHUAHUA: one female from one of three *O. leucogaster* taken about 55 kilometers south of Ciudad Juarez on the El Paso–Chihuahua City highway.

Fahrenholzia pinnata Kellogg and Ferris

Our specimens agree in detail with the description given for this species by Ferris (1922), except that in many of our specimens the toothlike process at the extensor-basal angle of the tarsus of the second leg is poorly developed and sometimes virtually absent. This is contrary to the condition shown in figures given by Ferris (1922, Fig. 104; 1951, Fig. 77) who shows a well developed process on the tarsi of both the second and third legs. In the western part of the United States and in Mexico *Fahrenholzia pinnata* is found on kangaroo rats (*Dipodomys*) and occasionally on pocket mice (*Perognathus*). Contrary to the condition reported here for *Hoplopleura hesperomydis,* nymphs are almost as frequent in occurrence as adults and all stages of *F. pinnata* were taken in every month of the year in the Santa Fe County collections.

RECORDS: BERNALILLO COUNTY: One male from one of two specimens of *Dipodomys ordii* (Woodhouse); LUNA COUNTY: one female from one of two specimens of *D. spectabilis* Merriam and one female from one of five specimens of *D. merriami* Mearns, taken about four miles west of Columbus; OTERO COUNTY: one female and one nymph from one of seven specimens of *D. merriami* taken about seven miles south of Alamogordo; SANTA FE COUNTY: 670 females, 261 males, 567 nymphs from 128 of 1128 specimens of *D. spectabilis baileyi* Goldman; 134 females, 31 males, and 92 nymphs from 71 of 2232 *D. ordii medius* Setzer; three females, five males, and 20 nymphs from 15 of 989 specimens of *Perognathus flavus flavus* Baird; CHIHUAHUA: seven nymphs from one of four specimens of *P. penicillatus* Woodhouse and four females, one male, and one nymph from five of 34 *D. merriami,* taken about 55 kilometers south of Ciudad Juarez, along the El Paso–Chihuahua City highway.

Neohaematopinus citellinus Ferris

Our specimens agree well with the original description of this species as given by Ferris (1942). The species is typically associated with ground squirrels (*Citellus*) and previously has been reported only from Arizona (Ferris, 1951). Except for the period of hibernation of the principal host, *Citellus spilosoma,* from December through March, we have taken specimens of *Neohaematopinus citellinus* during all months except August.

RECORDS: BERNALILLO COUNTY: 19 females, 13 males, and 12 nymphs from one of 12 specimens of *C. spilosoma marginatus* taken on the University of New Mexico golf course; SANTA FE COUNTY: 78 females, 18 males, and 36 nymphs from 33 of 326 *C. s. marginatus;* a single female from one of nine *C. variegatus grammurus* (Say); one female and one male from two of 134 *Cynomys gunnisoni zuniensis* Hollister; CHIHUAHUA: four females, four males, and 15 nymphs from a specimen of *Citellus tereticaudus* (Baird) taken about 55 kilometers south of Ciudad Juarez on the El Paso–Chihuahua City highway.

Neohaematopinus laeviusculus (Grube)

The usual hosts of this species are ground squirrels (*Citellus*), although it also occurs on prairie dogs (*Cynomys*). Our specimens agree well with the description given by Ferris (1923). The species ranges throughout western North America from Alaska to Mexico (Ferris, 1951).

RECORDS: BERNALILLO COUNTY: Four females and three males from a specimen of *Citellus variegatus grammurus* (Say) in June; SANTA FE COUNTY: three females, 11 males, and nine nymphs from two of nine *C. v. grammurus* taken during April and August.

Neohaematopinus marmotae Ferris

According to Ferris (1951) this species of Anoplura is distributed throughout the Rocky Mountain area of the United States and is confined to species of marmots (*Marmota*).

RECORD: SANTA FE COUNTY: 134 females, 98 males, and 222 nymphs from six (all infested) *Marmota flaviventris obscura* Howell collected during August.

Neohaematopinus neotomae Ferris

Except for a few intraspecies variations, our specimens agree with the original description given by Ferris (1942). In many of our specimens we have found the sternal plate of the thorax relatively longer and much more posteriorly narrowed than shown in the figure by Ferris (1942, Fig. 40-A). Regardless of modification in general shape, however, each postero-lateral corner forms a distinct point. In some of our specimens we have observed a great reduction in size and strength of the spinelike seta of the first segment of the antenna, this reduction having been reported previously by Ferris (1942) for specimens taken at White Sands, New Mexico. From our collections, it becomes apparent that the variation is intraspecific in nature, since some New Mexico specimens have this seta strongly developed as shown in the figure by Ferris (1942, Fig. 40-C), while other specimens have only a weakly developed unspecialized seta on the first antennal segment. Our specimens show all degrees of development from the virtual absence of a seta to the presence of a specialized and highly sclerotic spinelike seta. *Neohaematopinus neotomae* is known to be in the southwestern part of the United States and in Mexico. It occurs characteristically as an ectoparasite of various species of wood rats (*Neotoma*). Nymphs and adults of both sexes have been taken during every month of the year except June when only females have been found.

RECORDS: BERNALILLO COUNTY: five females, four males, and four nymphs from one *Neotoma micropus canescens Allen;* one nymph from one *N. mexicana* (Baird); 21 females, 16 males, and 54 nymphs from five out of nine *N. albigula albigula* Hartley; SANTA FE COUNTY: one female, one male, and three nymphs from three of 2232 *Dipodomys ordii medius* Setzer; 179 females, 60 males, and 115 nymphs from 134 of 981 *N. albigula albigula* Hartley; one female and three nymphs from one of nine *N. mexicana fallax* Merriam; 72 females, 18 males, and 93 nymphs from 51 of 579 *N. micropus canescens.*

Neohaematopinus pacificus Kellogg and Ferris

This species, typically ectoparasite on species of western chipmunks (*Eutamias*), has previously been reported only from California (Ferris, 1951).

RECORD: SANTA FE COUNTY: four females from three of 88 *Eutamias quadrivittatus quadrivittatus* taken during August and September.

Polyplax auricularis Kellogg and Ferris

Our specimens agree well with the description given by Ferris (1923) except that in some males the pseudopenis is not terminally pointed as shown in the figure by Ferris (1923, Fig. 141-D) but is terminally blunt, and in a number of specimens the very posterior tip appears to be notched or weakly incised. This species has been reported from Alaska to Mexico in the western part of North America and is usually found on species of deer mice (*Peromyscus*) and grasshopper mice (*Onychomys*). We have obtained collections of *P. auricularis* in central and northern New Mexico during all months of the year except July and December. For the most part, individual rodents have been found infested with no more than four

or five lice, but a few rodents have been found with much heavier infestations. For instance, one specimen of *Peromyscus maniculatus* (Wagner) from the Sandia Mountains in Bernalillo County was infested with 30 females, 27 males, and 12 nymphs.

RECORDS: BERNALILLO COUNTY: two females from one of five *Onychomys leucogaster* (Wied); 101 females, 62 males, and 29 nymphs from 29 of 65 specimens of *Peromyscus maniculatus* (Wagner); one female from one of seven *P. nasutus* (Allen); LUNA COUNTY: two females and three males from one of two *O. leucogaster* (Wied); two females from one of two *P. maniculatus* (Wagner); all taken two to four miles west of Columbus; SANDOVAL COUNTY: two females, one male, and one nymph from two of three *O. leucogaster* (Wied); SANTA FE COUNTY: seven females and one male from six of 405 specimens of *O. leucogaster pallescens* Merriam; 30 females, five males, and two nymphs from 15 of 445 *Peromyscus maniculatus rufinus* (Merriam); nine females and three males from two of 21 *P. nasutus nasutus* (Allen); one male from one of 662 *P. truei truei* (Shufeldt).

SUMMARY

From 1948 to 1954, over 3600 lice (Hoplopleuridae) were collected in New Mexico and northern Mexico from 8,784 examinations of 22 rodent species. Observations on the 11 species of Anoplura include: (1) geographic and host distribution; (2) seasonal abundance of females, males, and nymphs; (3) brief notes on morphological variation.

ACKNOWLEDGMENT

Much of the Santa Fe County field work was done by Mr. Huey C. Owen and Mr. John S. Adams under the direction of Dr. Robert Holdenried. Specialists who generously made mammal identifications were: Dr. Robert T. Orr, Dr. E. Raymond Hall, and Dr. Donald F. Hoffmeister. Identifications of representative specimens of lice were confirmed by Dr. Harry D. Pratt.

Acknowledgment is made to Dr. David H. Johnson, United States National Museum, for determination of species of rodents collected in Mexico. Acknowledgment is also made to Mr. Larry Gordon for aiding in the field work and to Mr. Hilliard Lewis, who assisted in field collecting and in making species determinations of many of the rodents taken in Bernalillo County, New Mexico.

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ON WHALE-LICE (AMPHIPODA: CYAMIDAE) FROM THE CALIFORNIA GRAY WHALE, *ESCHRICHTIUS GLAUCUS**

D. E. HURLEY** AND J. L. MOHR†

Because of the international restrictions on the taking of the California Gray Whale, there are few recent records of the parasites of this cetacean. However, under the international whaling convention eskimos are permitted to take any species for their own use and on August 10, 1954 took a female about 3 miles off Barrow, Alaska. Although, with characteristic eskimo efficiency in processing such catches, the villagers had scattered the parts of the whale beyond hope of identification by the time one of us reached Barrow a half day later and although the eskimos characterized it as both a humpback and a finback whale (two very dissimilar leviathans), Mr. William Maher, of the Museum of Vertebrate Zoology, University of California, and Dr. Raymond M. Gilmore, of the U. S. Fish and Wildlife Service, La Jolla, on the basis of Maher's notes and photographs as well as photographs of Dr. M. E. Britton, Geography Branch, Office of Naval Research, have been able to identify the animal as *Eschrichtius* (or *Rhachianectes*) *glaucus*.

Mr. and Mrs. Pete Sovalik and Mr. Daniel Cohen took several hundred cyamids mostly from the axillary region and from the skin about the genital region along with a few deeply imbedded barnacles, *Cryptolepas rhachianecti*. No parasitic copepods were noted. The cyamids and barnacles were presented alive (to J. L. M.) with some bits of whale skin in seawater at the Arctic Research Laboratory, Point Barrow. Some of the cyamids were fixed immediately, part in formol/seawater and part in Bouin's fluid. The remainder were stored in a household refrigerator in seawater which was changed daily. Each day individuals dead or appearing feeble were fixed, until the tenth day, when only a few were motile, and all survivors were preserved.

The whale louse fauna tends to confirm the identification of the host as a gray whale and brings up several significant problems. Three species of cyamid were found on this whale. Two of these are, so far as is known, specific to the gray whale. One of these, *Cyamus scammoni*, which is unique in having corkscrew type gills, was described from the gray whale in Californian waters by Dall in 1872. It has since been recorded from Korean (Andrews, 1914) and Southern Kamchatkan (Zenkovitch, 1934) waters and more recently from British Columbia (Margolis, 1954).

The second species, here re-described, in its ventral spination and gill-structure, is unlike anything we had previously noted. Mr. Leo Margolis, Pacific Biological Laboratory, Nanaimo, drew our attention to the possibility of its being *Cyamus kessleri*, described by Brandt in 1872, and then by Lütken (1873). It has not been recorded since. Margolis (personal communication) comments as follows: "The original specimens of Brandt's species were collected in the Bering Sea (presumably near Kamchatka) but the host is uncertain—usually stated as *Balaena* sp.

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Allan Hancock Foundation Contribution Number 184.

I have a very strong suspicion that the species discussed in your letter is *Cyamus kessleri*. . . . Since the gray whale occurs off Kamchatka, this whale probably was the host of the original specimens."

The third species, in some respects the most interesting of the three, occurs in numbers approximately equal to those of *Cyamus scammoni*, together about 5% of the infestation. It is *Cyamus ceti*, so far known only from the bowhead or Greenland right whale, *Balaena mysticetus*.

Generally speaking cyamids are host specific. This is not unexpected considering the circumstances of their life-history. The young whale-lice are released from the female broodpouch as miniature adults; there is no free-swimming stage. The adults have those appendages which are adapted for swimming in other amphipods differently modified, often greatly reduced, the peraeopods or walking legs being essentially paired peavey hooks which dig deeper into the whale skin as currents (or collectors) tend to pull the cyamid body away from the host. Accordingly, chances of transfer from one host individual to another, quite apart from any other restrictions of specificity, are limited to three general types of actual bodily contact. There is contact between adult and calf at birth and in suckling, between adults during courtship and copulation, and accidental or occasional contact through closeness in migration, in play and in attack upon one another. Of these only the last category is likely to account for transfer from one species to another. Dr. Raymond Gilmore has pointed out opportunities for contact between gray whale and bowhead in the Alaskan portion of the range of the former.

It is of particular interest that neither *Cyamus ceti* nor *C. kessleri* has been reported previously from the gray whale, especially in view of the great numbers of the latter in the present infestation. It is possible that they may have been missed by other collectors. *C. ceti* resembles *C. scammoni* (although the gills are notably different) while *C. kessleri* approaches the general outline of other *Cyamus* species. A collector looking for novel material might easily overlook *C. kessleri* because of its apparent lack of novelty. On the other hand, it is likely that *C. scammoni* occurs alone or as dominant form (as the presence of an alternate form would otherwise have been noted by Dall, Margolis, and perhaps by Zenkovitch [1934, 1937], although the reports of this latter author do not indicate close study of the cyamids), while infestation with *C. ceti* may indeed be uncommon.

Cyamus scammoni Dall, 1872

Dall, 1872: 281; Andrews, 1914: 260; Margolis, 1955: 129.

The cork-screw type gill of this species makes it very easy to distinguish from all other cyamids. Specimens constitute about 3% of whale-lice taken.

Cyamus ceti (Linne, 1754) Lamarck, 1801

Margolis, 1955: 130.

This species occurred in slightly smaller numbers than *C. scammoni*. The identification is quite certain; specimens agree very well with all descriptions, with specimens taken from bowheads captured at Barrow (coll. Ronald Coates and William J. Maher), and with specimens from the Stockholm Museum, also from *Balaena mysticetus*.

Cyamus kessleri Brandt, 1872

Brandt, 1872: 115–123, figs. 1–5
1872a: 675–683, figs. 1–5
Lütken, 1873: 258–260, pl. 2, fig. 3

Males up to approximately 15 mm long and 5 mm wide; females up to 10 mm long by 5 mm wide. Peraeon segment 1 fused to head; wider than head proper and swollen; head margins parallel. In male, peraeon segments 2 to 6 of approximately equal width, 3 slightly the widest; 7 distinctly narrower and subtriangular, notched above pleon insertion. Segments

2 and 7 longer than rest which are of approximately equal length. In female, segments 2 to 4 distinctly wider than rest and somewhat compacted together; 3 the widest.

Segment 2 in male distinct, anterior margin a little concave, posterior quite strongly convex; segment has swollen appearance. Segments 3 to 5 distinct, 3 a little directed anteriorly, 4 and 5 subrectangular.

There is a single pair of long cylindrical gills on each of segments 3 and 4, reaching forward approximately twice the distance from their bases to the head of the animal; there are 2 pairs of small cylindrical accessory gills ventrally, the posterior pair about half the width of their respective segments; the anterior pair shorter. The posterodistal angle of segments 3 and 4 is produced ventrally into a small cylindrical pseudo-accessory gill, half the size of the larger accessory gill. On the ventral surface of each segment near the bases of the accessory gills is a blunt spine process, in appearance quite similar to the small accessories.

Segment 5 has the anterior angles produced a little into a blunt, rounded process dorsally; a pair of blunt spines produced distally on the ventral surface. Segment 6 is slightly convex anteriorly, posteriorly concave; has a pair of blunt spine processes ventrally. Segment 7 has no distinct spines or tubercles ventrally in the male, except for a small pair of conical sexual appendages (Plate I, fig. 2), followed by the usual bilobed structure (Plate I, fig. 7) and the normal pleon structure.

In the female, segments 3 to 5 are much wider than 2; brood-plates are attached to segments 3 and 5 forming a large brood-pouch. There is one pair of small accessory gills on each of segments 3 and 4, the normal pair of large single gills is present; there is no pseudo-accessory gill. Segment 5 has a pair of strong inwardly-directed processes in the midline almost half the length and width of the segment itself; their contiguous inner margins are tipped with setae.

Segments 6 and 7 as in male except that segment 6 has four blunt tubercles ventrally; the anterior ones are the larger and are anteriorly directed; segment 7 has only the pleon structure, is otherwise naked except for two anterior prominences which are not distinct enough to be classified as tubercles or spines.

Antennae—First: Composed of 4 articles, 1 and 2 subequal, together about as long as head, 3 only fraction shorter than 2; all three have corona of small setae distally; 4 only about third length of 3, has terminal brush of setae. *Second:* About half length of first article of antenna 1; first three articles subequal, setae distally on 2 and 3; 4 half width and about third length of 3, has terminal brush of setae.

Mouthparts: Much as in *Cyamus boopis. Upper lip:* Appears to have one lobe setose, the other scabrous. *Maxilliped:* Has a very small palp rudiment in adults, sometimes practically none, only a few setae on plate and rudiment, a few minute surface spines on plate. In juveniles, the palp is absent in all specimens examined down to 2 mm. in length. *Maxilla 1:* Has 6 teeth on one side, 7 on other; palp rudiment not reaching base of teeth. *Mandibles:* Have scabrous accessory plate and molar process.

Gnathopods—First: Much smaller than second. In male: basos rectangular, stocky, width half length, third width and quarter length of 2; 4 subtriangular, short anterior free margin has small blunt lobe; posterior convex, length about two-thirds basos. Carpus as long, ovate, as wide as basos. Propod ovate, as long as basos, greatest width about three-fifths length; anterior margin strongly convex, posterior has strong blunt tooth about third, remainder concave and narrowing to stout curved dactylos which is three-fifths propod length. Female: generally similar but propod has a single weaker tooth about the middle of the posterior margin which is less concave distally. *Second:* Of 5 articles, the first slightly larger than second with flange on anterior margin; second ovate and not distinctly marked off from small third; propod greatly expanded, width almost half length; posterior free margin has 2 blunt teeth, is about third length of slightly convex anterior margin. Short curved dactylos as long as free posterior margin. *Peracopods:* 5 to 7 generally similar; quite long; of 6 articles; the propod stout and subrectangular, posterodistally has small lobe; anterodistally somewhat fused to small squarish ischium. Merus as long as basos, has narrow insertion, but anterior margin produced in squarish lobe almost as wide distally as long. Carpus as wide as merus, subsquare. Propod twice as long, anterior margin a little convex, posterior straight, narrowing to base of stout curved dactylos which is two-thirds propod length.

Dissections and other specimens in Allan Hancock Foundation, University of Southern California; representative series in U .S. National Museum.

As Margolis (personal communication) has suggested, this species corresponds to Brandt's *Cyamus kessleri.* The specimens agree very well with Brandt's figures (and the male, in general outline, far better than with Lütken's); the only discrepancies are in the points which Brandt omits: notably the spine-processes on the male

third and fourth segments ventrally, and the 2 pairs of spines on the female sixth segment ventrally (Brandt figures only 1 pair).

In these details, however, the specimens agree very consistently with Lütken's figures. Likewise they support his statement that there are not 3 accessory gills but that the third of Brandt's gills is really a process of the anterodistal angle of the segment. The term pseudo-accessory is used for this. Lütken illustrates the female sixth segment as shown herein (Plate II, fig. 3). There seems to be little doubt that we have here Brandt's *Cyamus kessleri,* and that, as Margolis has suggested, the characterization of the host as *Balaena* by previous authors may be in error.

The mouthparts of this species are very similar to those of *Cyamus boopis.* In a previous paper (Hurley, 1952), the senior author expressed the opinion that *Paracyamus* should be considered a distinct genus on the basis of the constancy of the maxilliped palp degeneration in *Cyamus boopis.* Margolis (1955) has shown that the constancy found in *boopis* is not present in certain other species (*C. erraticus* and *C. catodontis*). This, of course, destroys the force of the argument from *C. boopis;* accordingly, on the basis of his evidence, we have no hesitation in accepting *Paracyamus* as a synonym of *Cyamus.*

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Explanation of Plates

Plate I

Cyamus kessleri Brandt, 1872

Figure 1. Adult male. Dorsal view.
Figure 2. Adult male. Ventral view, segments 2–7.
Figure 3. Gnathopod 1, male.
Figure 4. Gnathopod 2, male.
Figure 5. Peraeopod 5, male.
Figure 6. Gnathopod 1, female.
Figure 7. Sexual appendages, male.

Plate II

Figure 1. Adult female. Dorsal view.
Figure 2. Adult female. Gills on segments three and four.
Figure 3. Adult female. Ventral surface of segments 5–7.
Figure 4. Antenna 1, male.
Figure 5. Antenna 2, female.
Figure 6. Upper lip.
Figure 7. Lower lip.
Figure 8. Mandible, male.
Figure 9. Mandible, female.
Figure 10. Maxilla, 1.
Figure 11. Palp of maxilla 1.
Figure 12. Maxilla 2.
Figure 13. Maxilliped.
Figure 14. Sexual appendage.
Figure 15. Pleon.

PLATE I

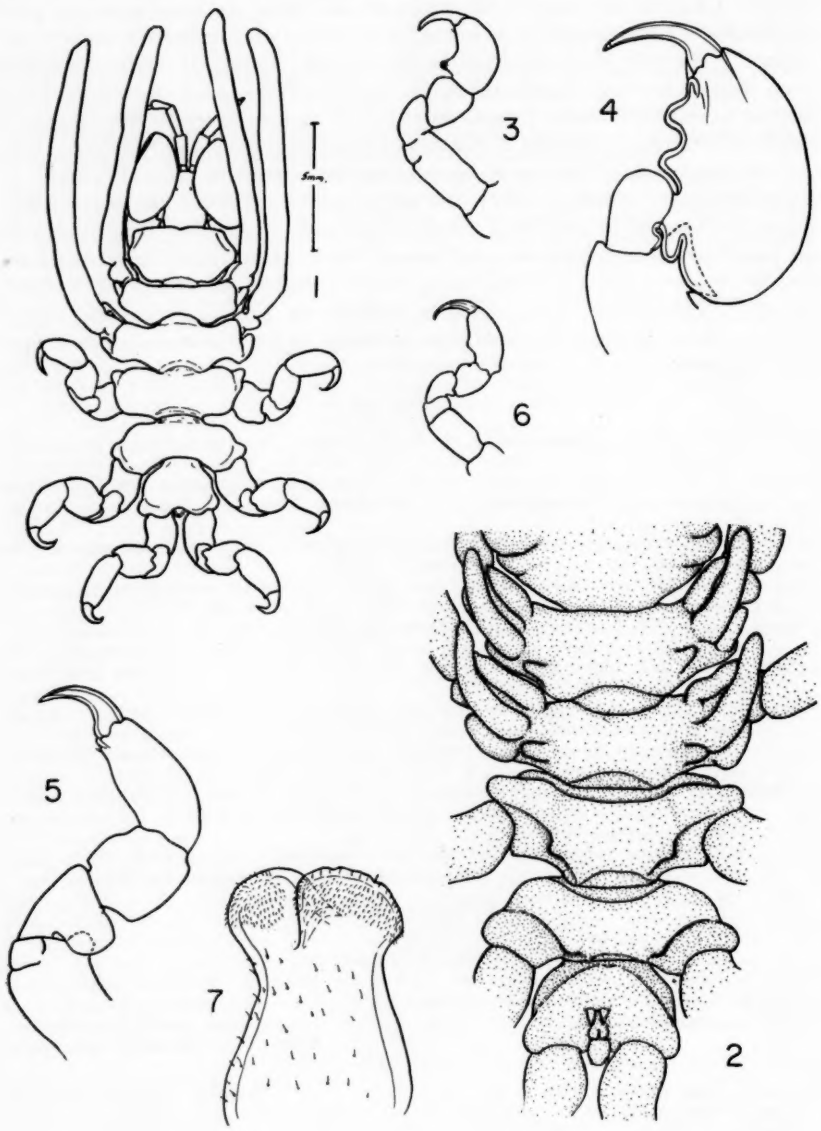
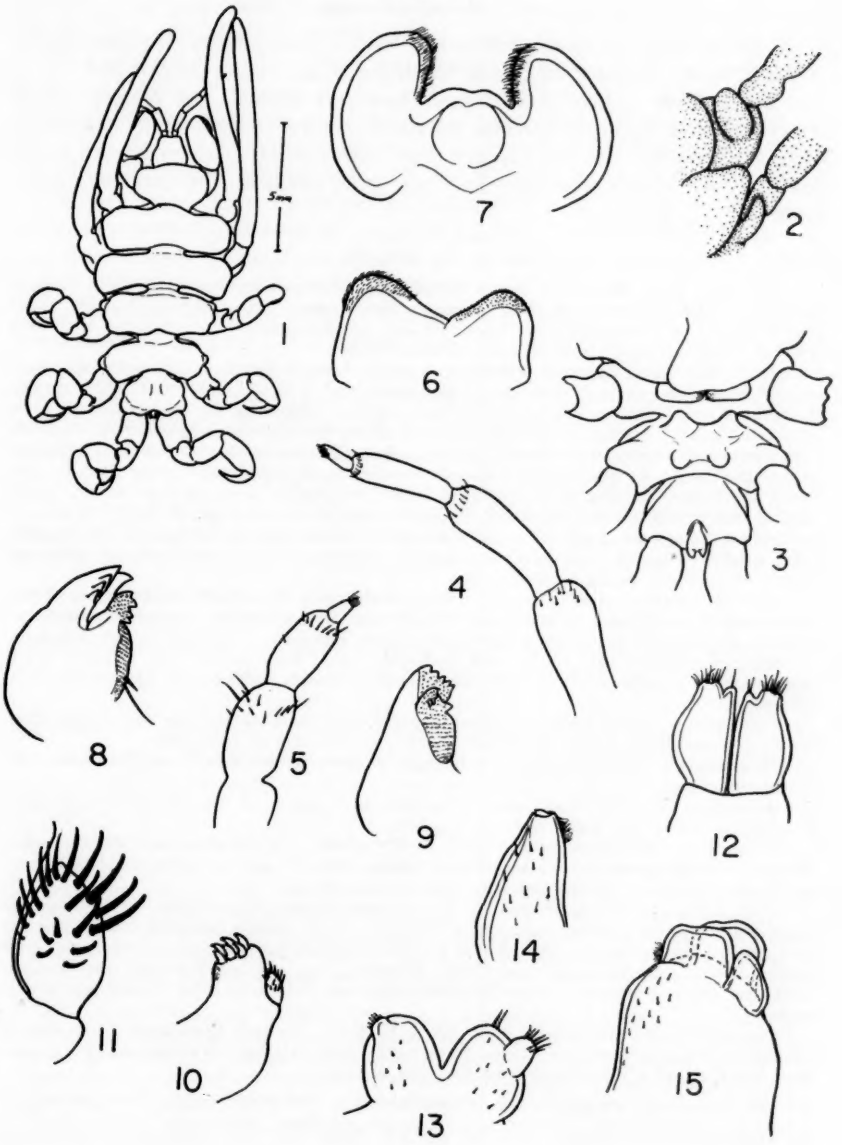


PLATE II



TWO NEW MESOZOA FROM THE PACIFIC NORTHWEST*

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Mesozoa from 8 octopuses, 2 taken near Friday Harbor in the San Juan Islands of Puget Sound, Washington, 5 from South Bay, Cape Arago, Oregon, and 1 from near Fossil Point in Coos Bay, Oregon, have been studied. All 8 were reddish octopuses belonging to the complex commonly termed *Octopus apollyon* (Berry) or *O. hongkongensis* Hoyle. The taxonomic status of this common octopus is still doubtful. All 8 were parasitized by 2 species of mesozoa, both heretofore undescribed.

Conocyema deca n. sp.
(Fig. 1, Fig. 2*a–h*, Fig. 3*b*)

Diagnosis: Small dicyemids closely resembling *Conocyema adminicula* McConn. 1949 in appearance but with 6 metapolar cells. Total number of somatic cells typically 23 or 24. Axial cell bluntly rounded anteriorly, not branching into the metapolars. Infusoriforms with very large urn cells and small inconspicuous refringent bodies.

Description: Small dicyemids mostly not exceeding 1 mm in length; total number of somatic cells 23 or 24 (4 propolars, 6 metapolars, 2 parapolars, and 11 or 12 trunk cells); trunk slender, of nearly uniform diameter throughout; cephalic swelling comprising the calotte plus the parapolar cells, inflated, sharply set off from the trunk giving the organism a hammerhead or mushroom appearance; calotte more heavily ciliated and deeply staining than rest of body, discoidal or even cup shaped, its cilia directed forward; metapolar cells considerably larger than the propolars which they surround or even project beyond anteriorly; parapolar cells larger, lighter staining than metapolars and containing irregular clumps of protein granules similar to those in some of the trunk cells; axial cell cylindrical, ending bluntly against the bases of the propolar cells; axial nucleus and most germinal elements commonly well forward; uropolar cells only slightly verruciform, charged with granules.

Vermiform larvae (Figs. 1*c* and 2*b*) stocky, mostly about 40 to 60 microns in length, though larger ones up to 100 microns have been observed; calotte large, blunt, of about same diameter as trunk, somewhat darker staining; axial cell extends anteriorly to the bases of the propolars; no rudimentary second axial cell in well developed larvae; axial nucleus well forward, in advance of the 1 or 2 germ cells present; very young vermiform embryos with typical chromatin elimination.

The cephalic swelling becomes inflated in young individuals shortly after their escape from the parent nematogens, during the period of active proliferation of germ cells.

Rhombogens do not show budding or amitotic divisions of their somatic nuclei characteristic of many dicyemids.

Infusorigen (Fig. 1*f*) usually small or medium sized, typical of those of most small dicyemids, giving rise to only a few embryos at a time.

Infusoriform (Fig. 2*c–e*) usually about 35–38 microns long, differing from those of other dicyemids in having smaller, less conspicuous refringent bodies and in having the 4 cells comprising the contents of the urn unusually large and conspicuous.

Host: Octopus apollyon (Berry) ? There is some doubt as to whether this is the proper name to apply to this octopus, and as to whether or not the reddish octopuses found along the west coast of North America from Alaska to Point Conception actually all belong to the same species. Those examined from Morro Bay, Monterey Bay, and San Francisco Bay in California had different species of mesozoa, none in common with those from Oregon and Washington.

Type specimens: Syntypes on Slide #4, J. L. Mohr, Hancock Foundation, University of Southern California, from an octopus taken near Friday Harbor, Washington. Paratypes: other slides #1–12 a series made from the same octopus.

This species is unique in the possession of 6 metapolar cells. It raises again the question of the validity of genus *Dicyemodeca* Wheeler, 1897, based solely on

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this character. Wheeler named this genus for dicyemids found near San Diego, California possessing this character, but not otherwise described. The writer (1949) after several years study of dicyemids from the same region concluded that the genus had been founded upon aberrant individuals belonging to other genera. The discovery of a species exhibiting this character, but in a different geographical region and in a species of octopus not known to occur near San Diego, necessitates a difficult taxonomic decision.

Should this northern species be regarded as the same as that named by Wheeler? The evidence from what is known of the geographical distribution of octopuses and of mesozoa along the west coast indicates that they are probably different. If the two are not the same, should the name *Dicyemodeca* be retained for the generic designation with the type species *sceptrum* Wheeler, a nomen nudum? The fact that there is some evidence that *Dicyemodeca* was founded upon aberrant individuals of other genera should also be considered.

In form and larval development this species closely resembles *Conocyema adminicula* McConnaughey 1949. Both have the characteristic inflated truncated discoidal cephalic swelling, composed of the deeply staining calotte plus the parapolar cells, sharply marked off from the cylindrical trunk. In both, the uropolar cells are only slightly verruciform and no other verruciform cells are present. The inclosed vermiform larvae of both lack an abortive second axial cell and tend to have the axial cell nucleus well forward in advance of the axoblasts or germ cells. Both species cling to the host kidney tissue by the entire flattened face of the calotte, establishing a more intimate contact than is the case with most dicyemids, and there is some evidence that they may sometimes digest host cells in the inflated cells of their calottes.

Because these peculiarities set these two species sharply apart from other known dicyemids it seems best to regard them as congeneric in spite of the difference in composition of the calotte and the absence of branching of the axial cell in the present species. As the generic name *Conocyema* van Beneden 1882 antedates *Dicyemodeca* Wheeler 1897, this conclusion simplifies the problem by obviating any necessity for dealing with the latter except as a questionable synonym, and has the advantage of bringing together these two remarkable and obviously related species.

Dicyemennea abreida n. sp.
(Fig. 2*i*; Fig. 3*a* and *c–h*)

Diagnosis: Dicyemennea, mostly short and stocky, usually not over 1 mm in length, with broad cephalic swelling widest at the level of the parapolar cells; body generally tapering towards posterior end except in unusually long individuals; no verruciform cells; somatic cells 24–34, usually 28–30; axial cell ending between the bases of the metapolar cells; contents of axial cell commonly crowded into its widened anterior portion; protein reserve granules in trunk cells spherical.

Description: Vermiform stages mostly small, less than 1 mm in length (long individuals 2 or 3 mm have, however, been found); total number of somatic cells of vermiform individuals variable, usually 28 to 30 (range observed 24–34); calotte broad, conical, bluntly and smoothly rounded anteriorly in most specimens, not truncate, appearing clear in life compared to the granular parapolar and anterior trunk cells; stained specimens with triangular-looking areas of basophilic cytoplasm in the cells of the calotte, not quite filling each cell; metapolar cells about 1½ to 2× as large as propolars; trunk cells lightly to heavily granular; especially anteriorly; cilia short, uniformly distributed; no verruciform cells; irregular amitotic division or budding of accessory nuclei characteristic of somatic cells of rhombogens, including even the metapolar cells as well as trunk cells.

Vermiform larvae cylindrical, of nearly uniform diameter, becoming 90 to 120 microns long before escaping from parent nematogens; calotte tall, darker staining than trunk; prominent small "nebenkern" often present in many of the somatic cells of well developed larvae; axial cell ending between the basal ends of the metapolar cells, a small rudimentary second axial cell present just anterior to the axial cell proper (this small cell absorbed; no trace of it present in adult vermiform individuals as is characteristic in genus *Dicyemennea*).

Infusorigens large, prolific, usually only 1 or 2 present in a given rhombogen, and usually located in the anterior swollen portion of the axial cell.

Infusoriform about 35 microns long, bluntly rounded behind, each cell of the urn with 2 free nuclei and 1 inclosed germ cell; the paired refringent bodies large, normal in appearance.

Host: Octopus apollyon (Berry) ? This species has been found in company with *Conocyema deca* in all octopuses examined to date from Washington and Oregon.

Types: Syntypes on slide 0-1, 1, author's collection. Paratypes on other slides of the 0-1 series from the same octopus. These slides also contain *Conocyema deca* but the two species are so different that this should cause no difficulty.

ACKNOWLEDGMENTS

The writer wishes to thank Dr. John L. Mohr of the Hancock Foundation, University of Southern California, and Mr. Carl Emanuel for preparations of mesozoa from the San Juan Islands, Puget Sound, Washington. Dr. James Macnab of Portland State College and Dr. David L. Jameson of the University of Oregon kindly donated octopuses taken by themselves and their students on field trips to Cape Arago. Mr. George Barton and Mr. David Galatro also contributed octopuses taken in the Coos Bay region.

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Illustrations

(All drawings made with the aid of a camera lucida, from preparations in the author's collection or from living specimens.)

Explanation of Figures

Figure 1. *Conocyema deca* n. sp.

a) Vermiform stages, entire, low power to show general appearance and size. Those showing the propolar cells of the calotte darkly strippled were made from fixed preparations; others from life.

b) Calotte of mature nematogen. Cell outlines could not be made out but the 4 small propolar, 6 larger metapolar and 2 still larger parapolar nuclei can be distinguished.

c) Young vermiform larva in the axial cell of the same nematogen shown in (*b*).

d) Isolated cephalic swelling of a young nematogen in face view showing arrangement of the cells of the calotte and the parapolar cells.

e) Early stages in the embryology of the vermiform larva.

f) Infusorigen from a young rhombogen.

Figure 2. *a–h. Conocyema deca* n. sp.; *i. Dicyemennea abreida* n. sp.

a) Very young nematogen. The germ cells are just beginning active proliferation and the cephalic swelling is becoming inflated and well marked.

b) Well developed vermiform larva still inclosed in the axial cell of the nematogen shown in Figure 1*b*.

c) Living infusoriforms showing the very prominent cells of the urn, and the inconspicuous character of the refringent bodies.

d) Young infusoriforms at stage just prior to division of the free nucleus in each urn cell. Cilia have begun to grow out from the somatic cells of the hind portion of the body but have not attained nearly their full length. The nuclei of the anterior cells which will bear the refringent bodies are already modified and irregular in appearance. Parasagittal and frontal optical sections.

e) Mature infusoriform showing the cilia in the cavity of the urn, the long posterior cilia, the 2 free nuclei and the germ cell in each of the urn cells, and the relatively small size of the refringent bodies. Parasagittal optical section, slightly oblique. A small polar body lying nearby in the axial cell of the parent rhombogen, in which there are apparently 8 chromosomes, is also shown.

f) Mature infusoriform in parasagittal optical section, showing the glycogen storing cell above the urn and capsule cells.

g) Young infusoriform of about same stage as those shown in (*d*), slightly oblique parasagittal optical section through urn below the level of the refringent bodies, stained with Mallory's triple stain for connective tissue. An accumulation of protein reserve materials staining bright blue can be seen as scattered granules forming throughout the cytoplasm of the capsule cells of the urn.

h) Mature infusoriform, ventral view. The blue-staining material seen in the capsule cells in (*g*) has been absorbed by the cells in the urn causing them to stain uniformly intensely blue at this stage. The capsule cells now stain much more lightly than before.

i) *Dicyemennea abreida*, vermiform larva still inclosed in the axial cell of its parent nematogen. Note the small abortive second axial cell between the bases of the metapolar cells at the anterior end of the axial cell. This is subsequently absorbed leaving no trace in older vermiform individuals. This is the larva in the nematogen shown in Figure 3 (*d*).

FIGURE 3. *Dicyemennea abreida* n. sp. (except *b*)

a) Vermiform stages drawn from life to show general features of size and proportions. (*b*. 2 nematogens of *Conocyema deca* which happened to be in the same field).

c) Rhombogen from fixed preparation, drawn to same scale.

d) A smaller nematogen from a fixed preparation, more highly magnified.

e) Anterior end of a nematogen.

f) Young infusorigen from a rhombogen prior to onset of meiotic divisions of the spermatogonia.

g) Immature infusoriform. The blue staining reserve material in the capsule cells appears as rather large spheres in their cytoplasm.

h) Nearly mature infusoriform. The blue staining reserve material remaining in the capsule cells is concentrated closely around the wall of the urn. The urn cells themselves now stain rather intensely blue, and their nuclei have divided.

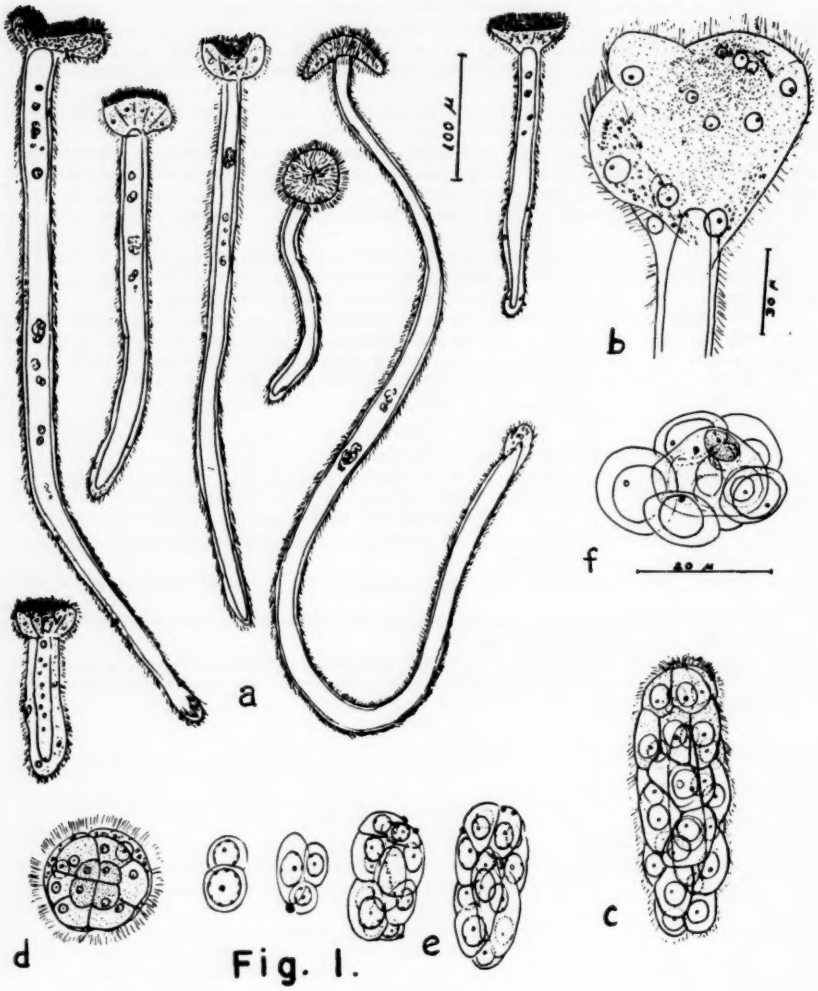

Fig. 1.

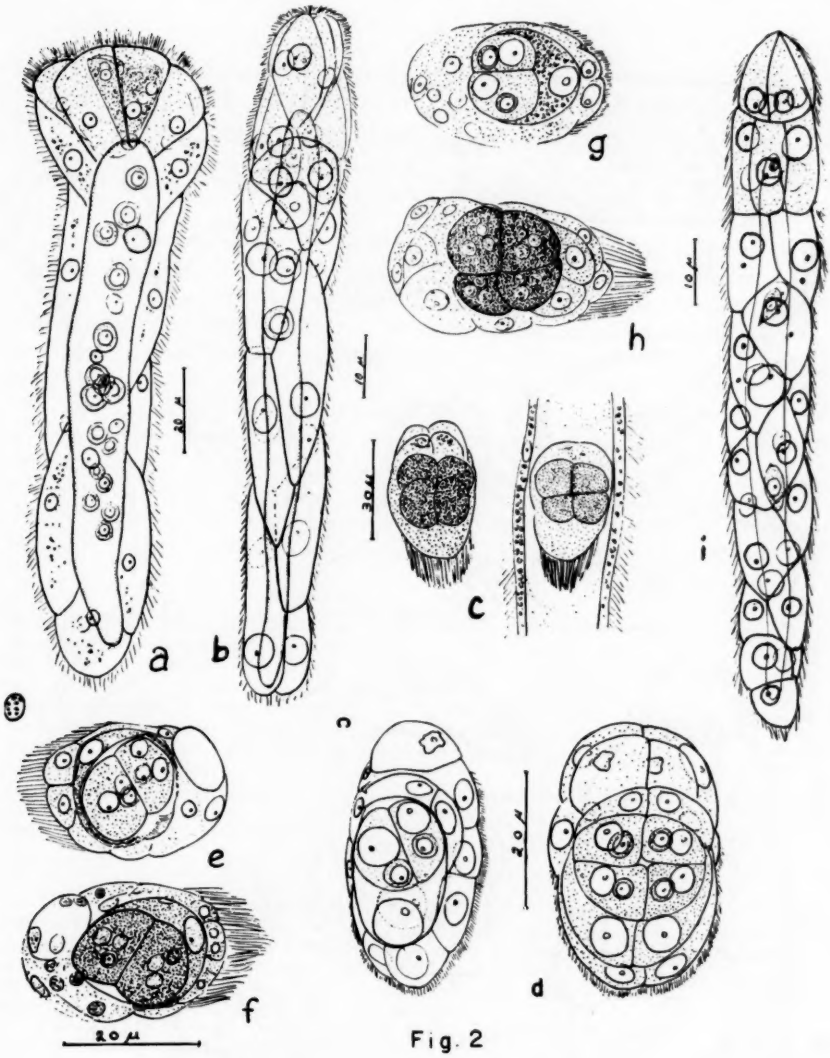

Fig. 2

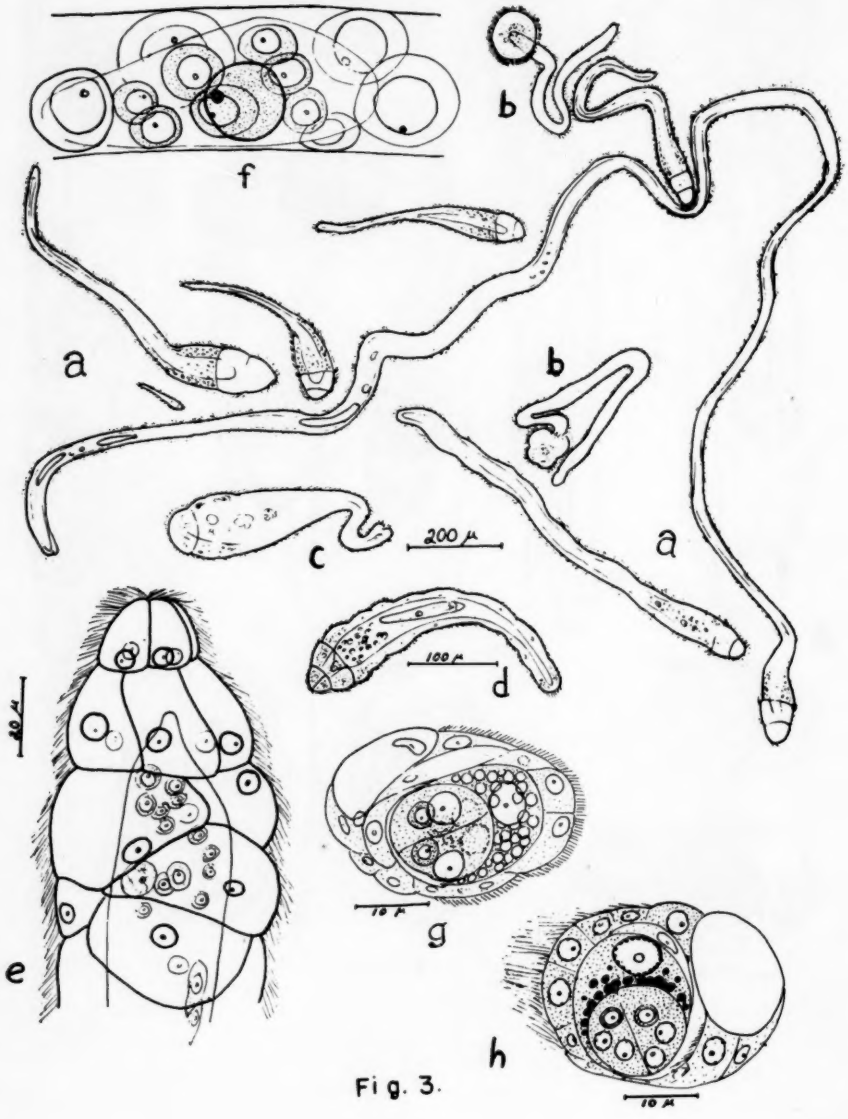

Fig. 3.

OOCHORISTICA CROTALICOLA, A NEW ANOPLOCEPHALID CESTODE FROM CALIFORNIA RATTLESNAKES

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Four stained and mounted specimens of a tapeworm belonging to the genus *Oochoristica* Lühe, were found to represent a species heretofore undescribed. The worms, from the small intestine of a Southern Pacific rattlesnake, *Crotalus viridis helleri*, from San Diego County, California, were collected and made available for study by Mr. Robert Schick. Additional specimens were obtained from a horned rattlesnake, *Crotalus cerastes laterorepens*, collected at Soboba Hot Springs, Riverside County, California, by Dr. John N. Belkin and Mr. William McDonald. The latter specimens consisted only of fragments of several worms, but were sufficiently well preserved to permit their identification with the species from the Southern Pacific rattlesnake.

All measurements are given in millimeters, unless otherwise indicated. Drawings were made with the aid of a camera lucida.

Oochoristica crotalicola n. sp.

Diagnosis: Anoplocephalidae; Gravid proglottid 0.94 long by 0.10 wide; mature proglottid 0.81 long; neck 0.90–1.00 long. Scolex 0.31–0.35 wide; suckers 0.11–0.16 in diameter. Testes 46–69 in number; cirrus sac 0.14–0.17 long by 0.09–0.10 wide. Ovary 0.17–0.24 wide; yolk gland 0.13–0.17 wide; eggs 25–39 microns in diameter; larval hooks 16–22 microns long. Genital pores irregularly alternating, opening in anterior fourth of proglottid margin. Ovary slightly toward poral side. Testes arranged laterally and posteriorly around ovary.
Hosts: Crotalus viridis helleri (Type); *C. cerastes laterorepens.*
Habitat: Small intestine.
Localities: San Diego County, California (Type); Soboba Hot Springs, Riverside County, California.
Holotype: U. S. Nat. Mus. Helm. Coll. No. 49094.

DESCRIPTION

The testes occupy most of the posterior half of the mature proglottid (Fig. A), extending anteriad to the level of the vitelline gland. The junction of vasa efferentia and vas deferens cannot be ascertained; the vas deferens first becomes visible as it widens considerably immediately anterior to the shell gland. It forms several tight coils in midfield, opposite the proximal end of the cirrus pouch, and narrows again as it enters the pouch where it forms a few coils inside the latter structure.

The female genital complex is situated in the proglottid center, close behind the level of the cirrus pouch. The compact, triangular vitellarium overlies the posterior margin of the ovary, between its 2 lobes. A short duct passes anteriorly from the yolk gland to an indistinct shell gland, overlying the mid-region of the ovary. The thin-walled vagina pursues a relatively straight course from the genital aperture to the ovary.

The egg capsule (Fig. C) is ellipsoidal in shape, with the onchosphere usually eccentrically located. Fixed (AFA), unmounted egg capsules measure 58 to 64 microns long by 45 to 51 microns wide. In cleared and mounted specimens the onchosphere is characteristically separated from its outer membrane by a relatively wide, clear area. In gravid segments the egg capsules fill most of the proglottid.

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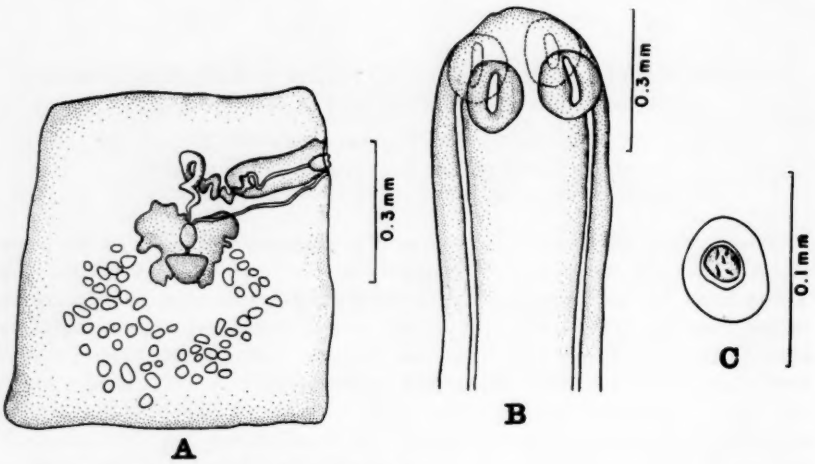

A. Mature proglottid; B. Scolex; C. Egg.

DISCUSSION

In having smaller eggs, *O. crotalicola* differs from *O. scelopori* Voge and Fox, 1950 and *O. anniellae* Stunkard and Lynch, 1944, both of which are recorded from California lizards. In addition *O. crotalicola* differs from *O. scelopori* in having a larger number of testes and a wider cirrus pouch, and from *O. anniellae* in having a larger ovary and a more lateral distribution of the testes.

O. crotalicola resembles *O. gracewileyae*, described by Loewen (1940) from *Crotalus atrox atrox*, in the diameter of the suckers, but differs from *O. gracewileyae* in having a smaller number of testes, and in the disposition of the testes in the mature proglottid.

O. crotalicola most closely resembles *O. osheroffi* Meggitt, described from the bullsnake, *Pityophis sayi*. However, the scolex of *O. osheroffi* is considerably wider than that of *O. crotalicola*. Study of Meggitt's (1934) figures of *O. osheroffi* reveals that the ovary is larger, the female genital complex is more centrally located, and the testes are more compactly arranged in that species than in *O. crotalicola*.

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ZYGOLAELAPS MADAGASCARIENSIS (ACARI: LAELAPTIDAE) A NEW GENUS OF MITES FROM MADAGASCAR

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Plague studies conducted in Madagascar in 1951–1952 by a United States Army Team involved the collection of ectoparasites from indigenous rodents and other small mammals. Among the ectoparasites collected is an undescribed laelaptid mite of unusual interest in that it possesses characters which relegate it to an intermediate position in relation to the genera *Gigantolaelaps* Fonseca 1938 and *Eubrachylaelaps* Ewing 1929. It is considered to be distinct enough to warrant the erection of a new genus which is described herein.

Zygolaelaps n. g.

Large, ovoid, spiny mites of subfamily Laelaptinae. Fixed chela of female toothed; moveable chela with inflated seta; long, strongly reflexed in male. Tectum subtruncate to lobiform. Deutosternum with 6 rows of teeth, 3–6 teeth per row. In female: sternal plate with 3 pairs of setae; endopodal setae slightly smaller than sternal setae; genito-ventral plate reduced, not expanded, coarsely reticulate, 1 pair setae; anal plate pyriform, 1 pair anal setae, 1 postanal seta; metapodal plates small, irregular. Holoventral plate of male entire, numerous setae. Stigmata lateral to coxae III; peritremalia extend posterior to stigmata. Dorsal plate entire, numerous setae in both sexes; covers entire dorsum in male but dorsum of female with wide posterolateral band of unsclerotized integument.

Type: *Zygolaelaps madagascariensis* n. sp.

Zygolaelaps very closely resembles *Eubrachylaelaps* Ewing 1929 and shares some characters with the genus *Gigantolaelaps* Fonseca 1938 as is shown in Table I. Furman (1955) clearly separates *Gigantolaelaps* from *Eubrachylaelaps* as follows: "Another genus which is very closely related to *Eubrachylaelaps* is *Gigantolaelaps* Fonseca 1938. It appears to differ, however, in the following characters: Tectum lanceolate; peritremalia not produced posterior to stigma; some anterior setae of dorsal plate much elongated; body size 2 to 4 times as great as *Eubrachylaelaps*." On the basis of these differentiating characters *Zygolaelaps* is related to *Gigantolaelaps* by virtue of size and heavy body pilosity and in addition their hosts share a similar habitat. *Zygolaelaps* differs from *Gigantolaelaps* in having peritremalia which are produced posterior to the stigmata and a tectum which is subtruncate to lobiform and in lacking the following characters peculiar to *Gigantolaelaps:* a median anterior projection of the sternal plate, elongate anterior setae of dorsal plate, and elongate setae on coxae II. *Zygolaelaps* is more closely related to *Eubrachylaelaps* as indicated by the similarity of the tecta and in sharing other characters contained in Table I. *Zygolaelaps* may be differentiated from *Eubrachylaelaps* in that the latter is approximately one-half the size of the former, body pilosity is much lighter and the body more ovoid (See Table I). In addition, mites of the genus *Eubrachylaelaps* have been collected only from cricetine mice in the arid regions of Western North America whereas mites of the genus *Zygolaelaps* were collected on an aquatic host, a shrew, in the Ethiopian zoogeographic realm.

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* Captain, MSC.

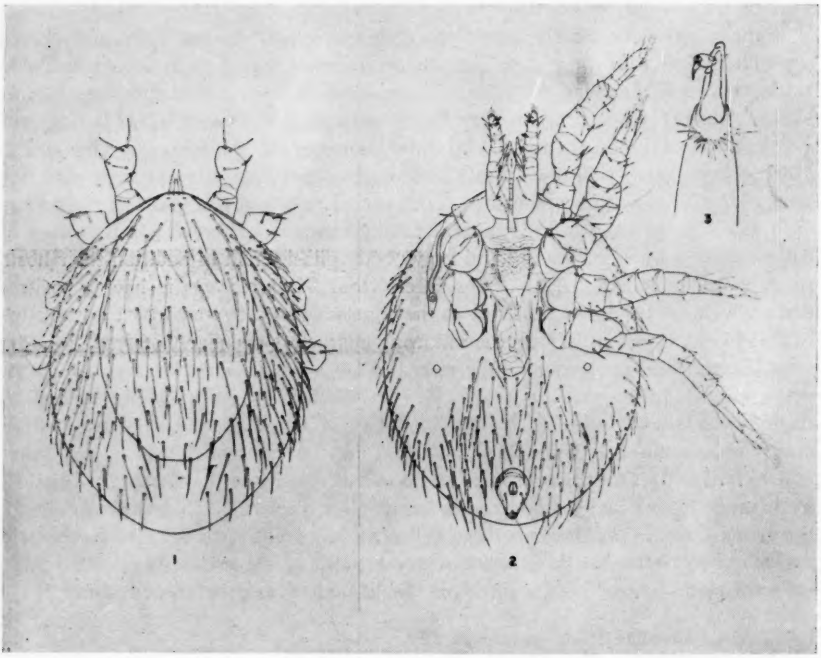
TABLE I.—Comparative Chart of Four Closely Related Genera of Laelaptinae

NO.	CHARACTER	ZYGOLAELAPS GEN. NOV.	GIGANTOLAELAPS FONSECA 1958	EUBRACHYLAELAPS EWING 1929	HAEMOLAELAPS BERLESE 1910
1	LENGTH OF IDIOSOMA EXCEEDING 1400 MICRONS	+	+	0	0
2	RATIO OF WIDTH OF IDIOSOMA TO LENGTH	5:7.8	5:6.9	5:6.6	5:7.7
3	BODY PILOSITY HEAVY	+	+	0	0
4	PRONOUNCED ANTERIOR PROJECTION OF STERNAL PLATE	0	+	0	0
5	SPINE-LIKE SETA PRESENT ON ONE OR MORE PAIRS OF COXAE	+	+	+	0
6	ELONGATE SETAE ON DORSAL APICES OF FEMORA	+	+	+	0
7	ANTERIOR SETAE OF DORSAL PLATE ELONGATE	0	+	0	+
8	SETAE OF COXA II LONGER THAN SETAE OF OTHER COXAE	0	+	0	0
9	MORE THAN 15 PAIRS OF SETAE ON UNSCLEROTIZED INTEGUMENT OF VENTER	+	+	0	0
10	PERITREMALIA PRODUCED POSTERIOR TO STIGMA	+	0	+	+
11	SOME POSTERIOR MARGINAL SETAE FEATHERED	0	0	+	+
12	TECTUM LANCEOLATE	0	+	0	0
13	NUMEROUS SETAE ON EXPANDED PORTION OF HOLOVENTRAL PLATE (MALES)	+	+ *	0	0
14	HABITAT OF HOSTS AQUATIC OR SEMIAQUATIC	+	+	0	+0

+= CHARACTER PRESENT

0= CHARACTER ABSENT OR NOT AS DESCRIBED

*G. BUTANTANENSIS HAS BUT FEW SETAE ON HOLOVENTRAL PLATE.



FIGURES 1–3. *Zygolaelaps madagascariensis*, female
FIGURE 1. Dorsum. FIGURE 2. Venter. FIGURE 3. Chela.

Zygolaelaps madagascariensis, n. sp.

Female (Figs. 1–3)

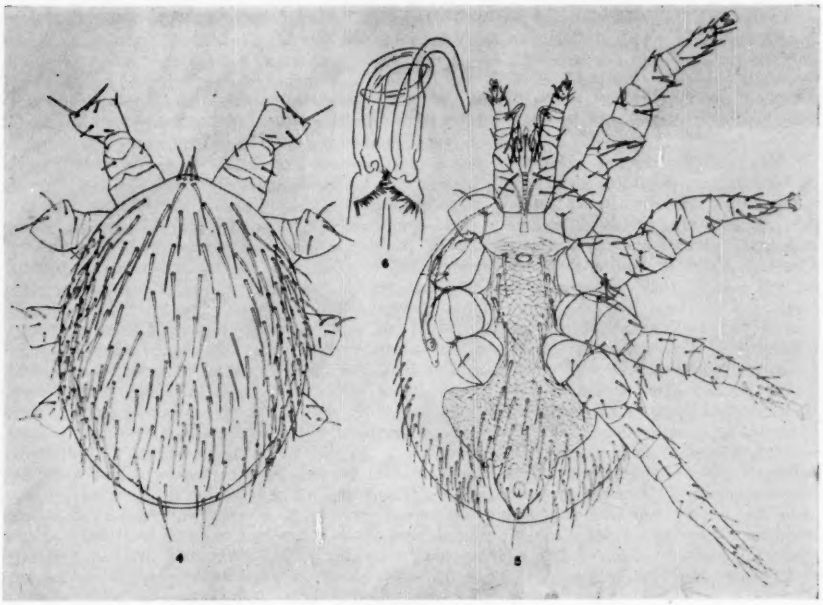
Idiosoma: 1540 microns long; 980 microns wide at widest point.

Dorsum: Dorsal plate oval; coarsely reticulate; does not cover entire dorsum; 1160 microns long, 650 microns wide; approximately 45 pairs of uniform setae; 2 small, slit-like pores near anterior margin. Unsclerotized integument of dorsum a wide, posterolateral band with approximately 75 pairs of setae.

Venter: Tritosternum feathered from bifurcation near basal segment. Presternal area reticulate; lightly sclerotized. Sternal plate reticulate; 170 microns long, 218 microns wide at narrowest point; anterior and lateral margins concave, posterior margin straight to slightly convex; first pair of setae on anterior margin, slightly smaller (125 microns) than second (158 microns) and third (145 microns) pairs; 2 pairs of slit-like pores roughly parallel to anterior margin. Setae of endopodal plates definitely smaller (102 microns) than those of sternal plate. Genito-ventral plate coarsely reticulate; not expanded; with 1 pair setae; 380 microns from posterior margin of genito-ventral plate to posterior margin of sternal plate, ratio of width to this length: 2: 2.5. Metapodal plates small, irregular shape. Anal plate pyriform; margin with reticulation of fine lines; paired setae 90 microns long, opposite posterior margin of anal pore; postanal seta 160 microns long. Non-sclerotized integument of venter non-reticulate; with approximately 60–70 pairs setae. Stigmata open near posterior margins of coxae III; peritremalia slightly sinuate, extend to middle of coxae II; posterior extension with small apical cleft and pore.

Legs: Legs robust; with caruncles and claws; coxae with moderately heavy setae; only coxa III bears spine-like seta; second pair of legs more robust than other legs. Setae on dorsal apices of femora I not greatly enlarged though larger than other femoral setae.

Gnathosoma: Deutosternum with 6 rows of teeth, 3–6 teeth per row. Gnathosomal setae small (48.6 microns) as compared with long medial hypostomal setae (110.2 microns). Also lateral (35.6 microns) and distal (55.1 microns) setae smaller than medial hypostomal setae. Hypostomal processes broad, becoming shear-like apically. Moveable chela untoothed; pilus dentilis with highly inflated proximal portion, distal portion strongly recurved, angulate. Fixed chela with 2 strong teeth. Row of fine setae at base of chelae. Hyaline tectum subtruncate to lobiform.



Male (Figs. 4–6)

FIGURES 4–6. *Zygolaelaps madagascariensis*, male

FIGURE 4. Dorsum. FIGURE 5. Venter. FIGURE 6. Chelae.

Idiosoma: 850 microns long; 600 microns wide.

Dorsum: Dorsal plate similar to that of female but covers almost entire dorsum; 815 microns long by 550 microns wide; approximately 75 pairs of setae similar in size and shape.

Venter: Holoventral plate entire; greatly expanded posterior to coxae IV; 400 microns wide at widest point; margin in proximity of anal plate somewhat irregular; coarsely reticulate; 19 pairs of setae exclusive of anal setae. Approximately 36 pairs of setae on unsclerotized portion of venter. Stigmata and peritermalia as in female.

Legs: Not unlike legs of female except that they appear to be more robust in proportion to overall body size. Spine-like seta on coxa III similar to those of female.

Gnathosoma: Setation similar to that of female but in addition oblique row of fine setae at base of chelicerae. Chelicerae long; broad at base; becoming subacuminate; toothless; strongly reflexed.

Holotype: A female collected by V. J. Tipton 69 Kilometers from Tananarive, Madagascar on the Tamatave road on 29 February 1952. Host: *Nesogale dobsoni* (USNM 294520). Deposited in the collection of the U. S. National Museum.

Allotype: A male, same data as above. Deposited in the U. S. National Museum.

Paratypes: One female and 2 males, same data as above. Deposited in the British Museum of Natural History.

Appreciation and thanks are tendered: Mr. Saburo Shibata of the Taxonomic Entomology Section of the United States Army 406th Medical General Laboratory in Tokyo, Japan for the preparation of Figures 1, 2, 4 and 5; Major Hugh L. Keegan of the same laboratory who was instrumental in making arrangements for the drawing to be done; Dr. Deane P. Furman, University of California, and Dr. Russell W. Strandtmann, Texas Technological College, for a critical review of the manuscript; and PFC Jack Boese, Fifth Army Area Medical Laboratory, for assistance in the preparation of Table I.

SUMMARY

Zygolaelaps madagascariensis, a new genus and species of parasitic mite from Madagascar is described. A comparative chart of 4 closely related genera of laelaptid mites is given.

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DIPLOSTOMULUM ELONGATUS N. SP. (TREMATODA), FROM A FRESHWATER FISH, *TRICHOGASTER FASCIATUS*, FROM INDIA

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Seventy specimens of a fresh-water fish, *Trichogaster fasciatus*, were examined in Lucknow, India, and 2 of these were found to harbor larval cysts of a digenetic trematode. It was determined that the forms were of the genus *Diplostomulum*, and inasmuch as they could not be identified as to species from existing material, they are described herein as new, and so named to designate the elongate, slender form and the comparative size.

The transparent cysts were found attached loosely to the mesentery inside the body cavity. The 2 specimens recovered differ greatly in size, but apparently they are 2 stages of the same species. While alive the larvae showed active movement, both inside and outside the cyst. The specimens were fixed in corrosive sublimate and mounted in balsam. All measurements given below are in millimeters, and were taken from the larger specimen.

Diplostomulum elongatus n. sp.
(Figs. 1–3)

Diagnosis: Cyst measures 1.02×0.67. Body of trematode elongate, aspinose, divided (but not well-marked) into flattened forebody slightly concave ventrally, 0.972 long by 0.558 wide, and small hindbody, 0.2 long. Comparatively small oral sucker somewhat ventral, muscular, 0.045 in diameter. Mouth opening sub-ventral. Lateral suckers appear as slightly muscular papillae, postero-lateral to oral sucker. Oral sucker leads directly into muscular pharynx, 0.03 in diameter; prepharynx absent. Short esophagus bifurcates into 2 intestinal ceca, running posteriorly well into hindbody. Muscular acetabulum, 0.07×0.04, rounded in living condition and transversely elongated when fixed, present in anterior to middle of body, 0.5 from anterior end. Hold-fast organ, much posterior to acetabulum near posterior end of forebody, longitudinally elongated in living state and rounded when fixed, 0.136 in diameter. Compact, elongated hold-fast gland, 0.132×0.03, seen with difficulty in living larva, stains deeply with carmine. Genital *anlagen*, consisting of irregular mass of undifferentiated cells, present between ceca in hindbody, somewhat posterior to hold-fast gland.

Primary excretory system and flame cells obscured by reserve excretory system and not seen. Reserve excretory system divisible into ventral ducts, forming major part of system, and comparatively few dorsal ducts. Excretory pore, present posteriorly, leads into small vesicle that divides into 2 main lateral excretory ducts running anteriorly. Lateral ducts with smaller ducts form excretory system ventrally. Two outer lateral ducts joined by transverse connection just posterior to oral sucker. Main excretory ducts joined in hindbody by 2 posterior transverse excretory ducts. Latter, dorsally, has apparently blind median process directed anteriorly, and connected to 2 lateral excretory ducts at same point. Two lateral excretory ducts joined in hindbody by 2 or 3 short ducts. In forebody, 2 medial lateral excretory ducts connected first by anterior transverse duct, and, proceeding anteriorly, several ducts join 2 lateral excretory ducts up to region of acetabulum. Several ducts loop and rejoin medial ducts on medial side. Anterior to acetabulum, 3 to 4 excretory ducts connect lateral ducts. From posterior side of transverse connection of outer lateral excretory ducts near oral sucker, small duct running posteriorly joins median excretory vesicle just posterior to intestinal bifurcation. Slightly posterior to opening, 2 inner lateral excretory ducts also join median excretory vesicle, latter running behind intestinal bifurcation to hold-fast organ. Posterior end of elongated, spindle-shaped median excretory vesicle passes ventral to transverse connection. Vesicle opening into transverse connection anterior to hold-fast organ not seen. Dorsal excretory ducts arise from 2 main lateral ducts in posterior region and branch, in hindbody, to form fine network, of which finer ducts regroup and form duct leading anteriorly into forebody, there branching dorsally to hold-fast organ to form fine network. Fine ducts

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arising from anterior face of latter network not traced. Many refractile excretory granules present in ducts. Calcareous corpuscles entirely absent.

Host: Trichogaster fasciatus, a fresh-water fish.

Locality: Lucknow, India.

Habitat: Attached to mesentery inside body cavity.

Type: In the helminthological collection, University of Lucknow, Lucknow, India.

DISCUSSION

A number of species of *Diplostomulum* have been described from other regions of the world but only 1 has been reported from India (Kaw, 1950). Hughes (1929) described 2 new species and gave a synopsis of the other species known. *D. elongatus* n. sp. differs from all other species in the position of the acetabulum (anterior to middle of the body) and in the presence of a well-developed hold-fast gland. It is most similar to *D. corti* Hughes, 1929, but differs from this species in the size, in the possession of a comparatively small hindbody, and in the absence of the bursa copulatrix. Further, in *D. corti,* as in other species of *Diplostomulum,* the hold-fast organ is present just posterior to the acetabulum, but in the present form these are widely separated, the distance between them being 0.295 mm, which is much more than the diameter of either of these organs. In *D. bufonis* Kaw, 1950, the acetabulum and hold-fast organ are widely separated, but *D. bufonis* differs from the presently described form, besides other characters, in the absence of the hold-fast gland.

The pattern of the reserve excretory system is essentially the same as in other species where it is known. However, the bifid excretory bladder, so well developed in other species, is almost entirely lacking in *D. elongatus* n. sp. Further, *D. elongatus* n. sp. possesses 2 lateral excretory ducts connected by means of a number of smaller ducts. In all other species of *Diplostomulum,* the dorsal medial excretory duct is almost of the same diameter as the other ducts; in the present form the median duct is comparatively very wide and long, and hence is called herein a vesicle. Because of the large size of the refractile granules within this vesicle, it appears to serve as an "excretory bladder" which stores the excretory products. Also, in *D. elongatus* n. sp., 2 networks of smaller ducts are present dorsally which are absent in other species. The medial excretory vesicle is connected, in *D. elongatus* n. sp., to the 2 lateral ducts in the region of the pharynx only, unlike the other species where several transverse connections are present in the middle of the body.

The calcareous corpuscles usually prominent in other species of *Diplostomulum* are entirely lacking in the present material, and it seems doubtful that they could have been overlooked.

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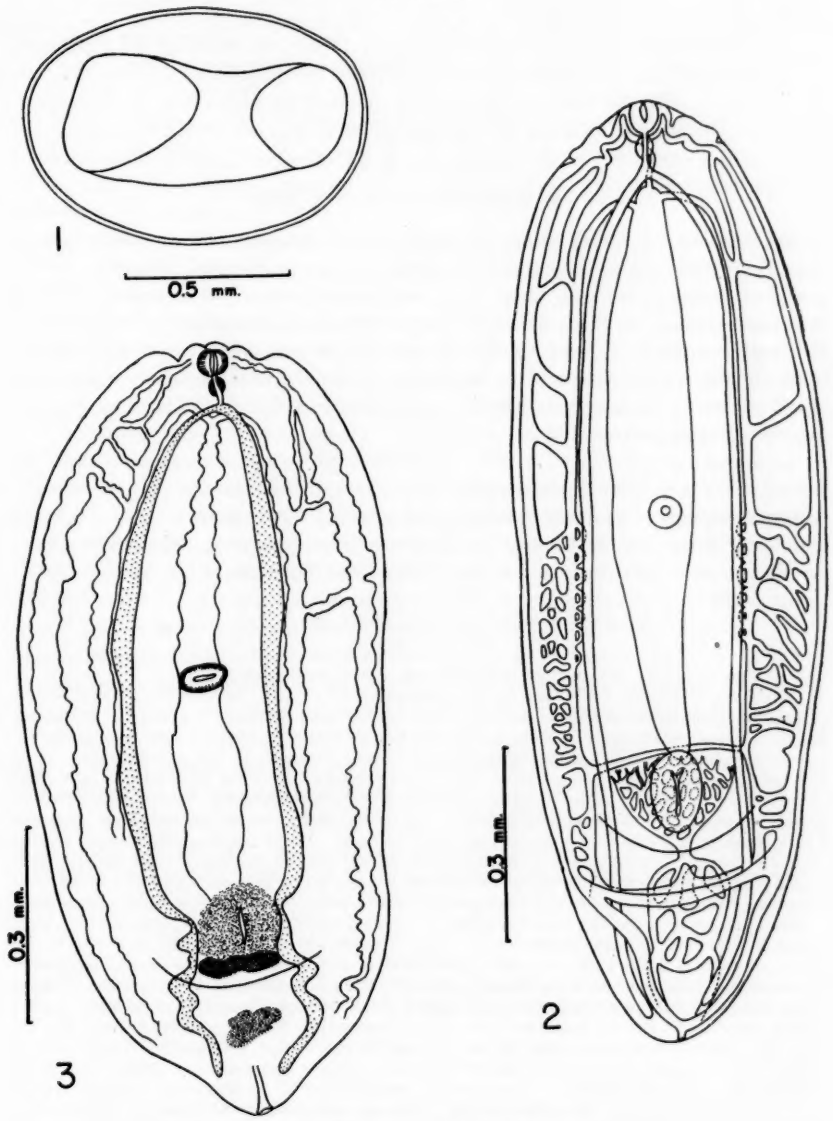
PLATE I



EXPLANATION OF FIGURES

FIGURE 1. *Diplostomulum elongatus* n. sp. Cyst with parasite inside.
FIGURE 2. *D. elongatus* n. sp. Living metacercaria, showing excretory system.
FIGURE 3. *D. elongatus* n. sp. Fixed metacercaria, showing morphology.

BILHARZIELLA LITTLEBI (BYRD, 1956), A BLOOD FLUKE (DIGENEA: SCHISTOSOMATIDAE: BILHARZIELLINAE) FROM THE CHESTNUT-SIDED WARBLER, *DENDROICA PENNSYLVANICA* (LINNAEUS)

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While making a survey of the worm parasites of resident birds at the Mountain Lake Biological Station, Mountain Lake (Giles County), Virginia, summer of 1947, a single male specimen of a blood fluke was removed from the mesenteric vein of the chestnut-sided warbler, *Dendroica pennsylvanica* (Linnaeus). The bird was shot and examined on June 30, and was one of the two specimens of that species taken throughout the summer. In removing it from the vein the worm was broken into 2 pieces. The two pieces became separated into 5 during the process of staining, dehydrating and mounting.

In a short abstract Byrd (1956) designated the species *Pseudobilharziella littlebi* n. sp. On examining the material more closely it was decided that this assignment was untenable since the specimen had a well defined cirrus pouch. Together with other characters the presence of the cirrus pouch makes it necessary to transfer the species from the genus *Pseudobilharziella* Ejsmont, 1929 to *Bilharziella* Looss, 1899.

Bilharziella littlebi (Byrd, 1956)
(Fig. 1, *a* and *b*)

* Unless otherwise indicated all measurements are in millimeters.

Body approximately 5.10* long by a maximum width of 0.19; anterior fifth more or less cylindrical, then becoming dorso-ventrally flattened for approximately one-half of total body length. Toward posterior end body again becomes somewhat cylindrical and tapers steadily to minimum width of 0.09 near posterior limit of testes. Posterior to testes body widens again and ends in short, spatula-like posterior part. Cuticle devoid of spines except for 3 or 4 rows of very fine spines which occur in cavity of both oral and ventral suckers. Oral sucker terminal, weakly muscular and measures 0.06 long and 0.10 wide; ventral sucker pedunculated, measures 0.10 in diameter and lies 0.34 behind anterior end of body. Mouth opening subterminal, antero-ventrally directed, leads through oral cavity into slightly dilated esophagus (0.21 long). Ceca spring from esophagus 0.13 in front of ventral sucker, pass posteriorly to region of seminal vesicle where one of them becomes lost from view (their reunion was not observed). Since only 1 cecum can be seen clearly from beginning of seminal vesicle throughout remainder of body fusion of both ceca probably occurs within area of seminal vesicle. Common cecum undulates gently in passing down body and ends approximately 0.16 before reaching caudal extremity. Gynecophoric canal appears to be absent: however, there is a thinning and flattening of body which begins at level of cirrus pouch and extends for approximately one-third of body length; lateral edges of this part of body have only a weak tendency to become infolded. There are 133 testicular masses; they begin close behind opening of sperm duct and continue posteriorly to within 0.40 of caudal end of body and are arranged more or less tandem although with some irregularities and overlappings. Testes small, spherical (0.03 in diameter) to transversely ovoid (about 0.03 long by 0.06 wide) bodies. From most anterior testis a narrow vas deferens leads forward to within 0.14 of ventral sucker where it dilates (maximum width of 0.08), turns posteriorly as seminal vesicle and gently undulates through 0.46 of body length before constricting abruptly. Posterior fourth of seminal vesicle enclosed by cirrus pouch (whether or not the entire seminal vesicle lies within the cirrus pouch could not be determined from the whole mount). Within cirrus pouch seminal vesicle remains dilated for about one-half length of "visible" pouch and then constricts abruptly into narrow, loosely coiled tube which leads into

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almost spherical pars prostatica (0.03 in diameter). Sperm duct leaves pars prostatica and passes postero-ventro-laterally for distance of about 0.13 before opening to outside near one margin of body, 0.66 behind ventral sucker. Remainder of cirrus pouch filled with prostatic gland cells (Fig. 1 *b*).

Host: Dendroica pennsylvanicus (Linnaeus).

Habitat: Mesenteric vein.

Locality: Mountain Lake (Giles County), Virginia.

Type and only specimen: U. S. National Museum Helminthological Collection, No. 55284.

Bilharziella littlebi appears to be the second member of the genus *Bilharziella* Looss, 1899, as currently constructed, and the only blood fluke to be described from a warbler.

Bilharziella littlebi differs from *B. polonica* (Kowalewski, 1895), the other member of the genus, in several important respects. The body of *B. littlebi* is longer and narrower, and is dorso-ventrally flattened only in the middle and caudal portions. There are 133 testes in *B. littlebi* as compared with 110 in *B. polonica.* The testes are arranged more tandem in *B. littlebi* and in so far as could be determined there is no testicular material anterior to the union of the two ceca. Minute spines are present in the cavity of both the oral and ventral sucker in *B. littlebi,* and the suckers appear to be closer together. The opening of the sperm duct to the outside is closer to the acetabulum in *B. littlebi.*

Bilharziella polonica has been reported from the European heron, *Ardea cinerea,* and from ducks belonging to several genera from Eurasia (Europe and Russia), as well as from the swan, *Cygnus olor,* which died in the Zoological Gardens, Washington, D. C. In reporting *Bilharziella littlebi* from a passerine bird, *Dendroica pennsylvanica,* we recognize the possibility of the record being due to a chance infection. The chestnut-sided warbler is a native American bird, and it ranges from its nesting locality (North from the southern reaches of the Appalachian Mountains) to Guatemala and Panama. The parasite could be endemic in almost any family group of birds within this large section of the United States, Mexico and Central America. It is not likely that the warbler is the normal host since the habits of the species do not include frequent contact with bodies of water of sufficient size and suitability for the transmission of blood flukes. It is more likely that the chestnut-sided warbler picked up a single cercaria accidentally at one of its "stops" during migration. The specimen containing the fluke was a fully grown male bird and was a member of a nesting pair. Although possible, it is not likely that the fluke was acquired in the immediate environs of the nesting site (Salt Pond Mountain, at 4000 feet in elevation).

Reference

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PLATE I

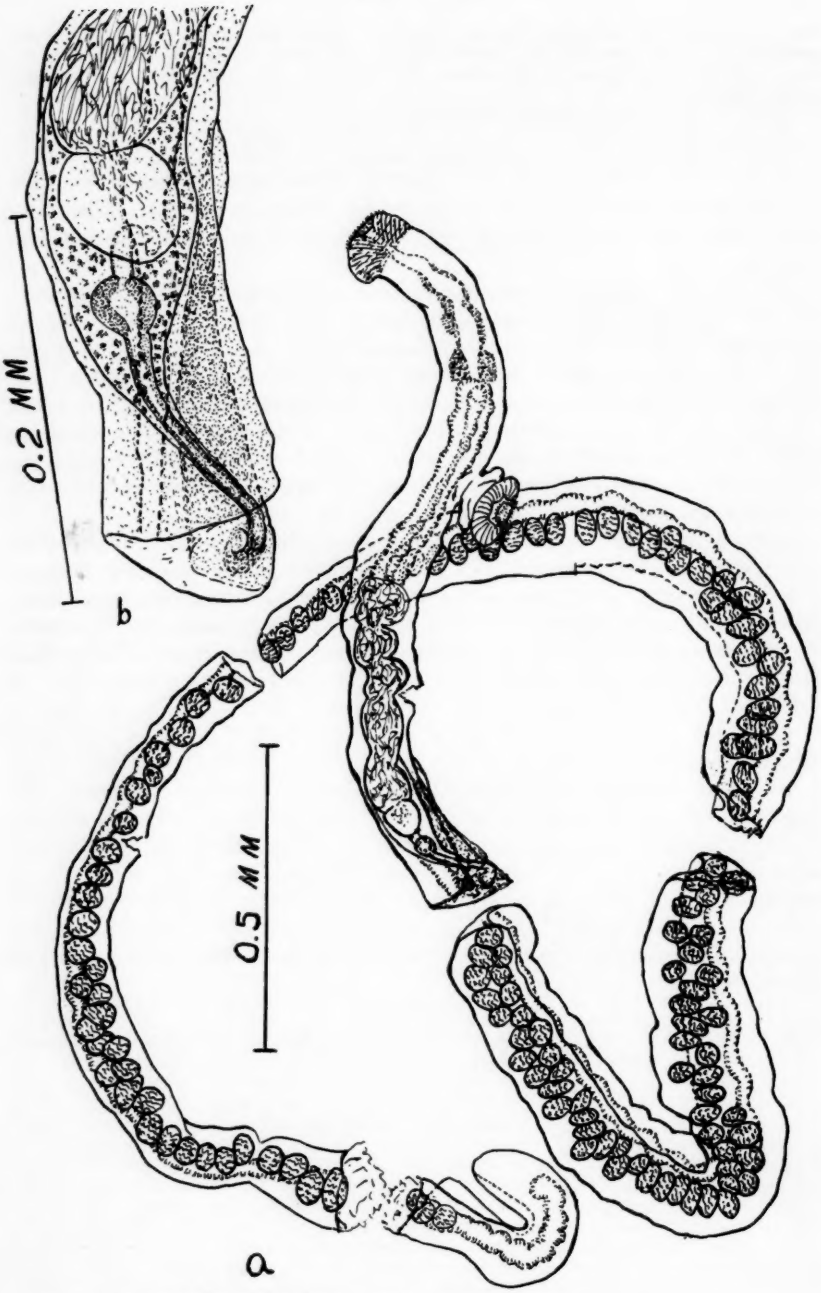

FIGURE 1. *Bilharziella littlebi* (Byrd, 1956). Camera lucida.
a. Lateral view of mounted specimen, arranged from the several pieces.
b. Enlarged view of the body at the level of the cirrus pouch.

OOCHORISTICA TANDANI N. SP. (CESTODA), FROM A SNAKE, *LYCODON AULICUS*, FROM INDIA

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A single specimen of cestode was obtained from a snake, *Lycodon aulicus*, killed in the house of my friend and colleague, Mr. B. K. Tandan, to whom my thanks are due for the material. On examination it proved to be a new species of the genus *Oochoristica* Luhe, 1898, and the species is named after the collector. Measurements appearing in the description following are in millimetres unless otherwise stated.

Oochoristica tandani n. sp.
(Figs. 1—2)

Diagnosis: Cestode about 90 long and 0.77 wide in region of gravid proglottids. Forty immature proglottids and short unsegmented neck present. Anterior proglottids 0.19 long 0.15 wide. Immature proglottids become progressively longer than wide; posterior proglottids 0.67 long by 0.384 wide. Mature proglottids, numbering less than 10, all longer than wide, 0.73–1.15 long and 0.42–0.52 wide. Gravid proglottids, 18 in number, usually 3 to 4 times as long as wide, measure about 2.3 long and 0.77 wide. Both ventral longitudinal excretory ducts and narrow dorsal ducts show small anastomosing branches; transverse excretory duct not seen.

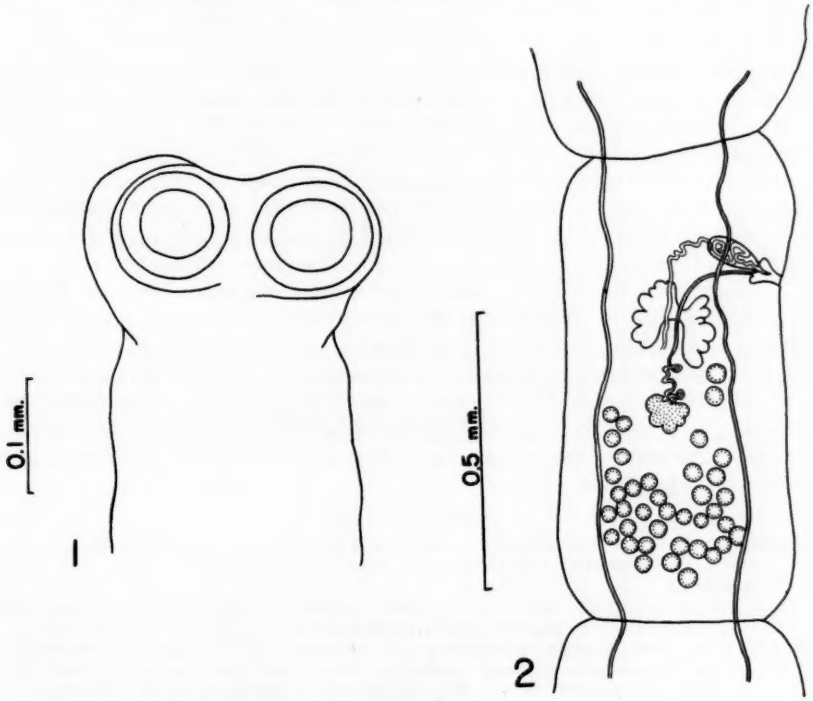

FIGURE. 1. *Oochoristica tandani* n. sp. Scolex.
FIGURE 2. *O. tandani* n. sp. Mature proglottid.

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Cuticle about 4 microns in thickness. Scolex small and unarmed, 0.15 long and 0.27 wide, not well marked off from the neck, with 4 rounded, muscular suckers, 0.10–0.13 in diameter. Genital pores, about ¼ from anterior margin of proglottid, marginal and irregularly alternate. Genital ducts pass between longitudinal excretory ducts ventral to nerve. Testes rounded, 37 to 45 per proglottid, 0.04–0.06 in diameter, and lie in one field posterior to female genital organs, though some reach to level of vitelline gland and may extend between longitudinal excretory ducts. Unarmed cirrus blunt distally, measuring 0.02 in diameter at base and 0.11 long when fully everted. Slightly elongate cirrus pouch, 0.09–0.1 long and 0.04–0.06 wide, extends beyond longitudinal excretory ducts across ¼ of proglottid. Cirrus pouch in mature proglottids thickly walled, surrounded by deeply staining nuclei. Vas deferens arises in region of female genital organs, curving towards and entering cirrus pouch after few coils. Ductus ejaculatorius slightly coiled inside cirrus pouch. Vesicula seminalis absent. Female genital organs present in middle of proglottid. Ovary bilobed, each lobe composed of 4–5 acini. Ovary measures 0.17–0.22 across in fully mature proglottids. Vitelline gland, lying posterior to ovary, rounded, slightly lobed, 0.09–0.11 in diameter. Shell gland 0.06 in diameter. Vagina arises from ootype, near shell gland, and runs parallel and posterior to vas deferens, opening posterior to male genital pore. Receptaculum seminis formed of widened vagina in region of ovary. Vagina not dilated terminally. Uterus develops as median sac with lobes. In fully gravid proglottids, eggs seen scattered in parenchyma filling entire proglottid and extending beyond longitudinal excretory ducts. Eggs 48–52 microns in diameter, with thick shell. Rounded onchosphere, 26–30 microns in diameter, possesses 6 hooks, each 13 microns long.

Host: *Lycodon aulicus.*
Locality: Lucknow, India.
Habitat: Intestine.
Type: In the helminthological collection of author, University of Lucknow.

DISCUSSION

Hughes (1940) revised the genus *Oochoristica* and gave a key to the 45 species that he recognised. Since then a number of species have been added from various parts of world both from mammals and reptiles (Loewen, 1940; Chandler, 1942; Stunkard & Lynch, 1944; Misra, 1945; Voge & Fox, 1950; Johri, 1950; Chandler & Melvin, 1951; Chandler, 1952; Hickman, 1954; Voge, 1954; Dollfus, 1954; Yamaguti, 1954; and Millemann, 1955). Dollfus (1954) described 3 new species and a new variety of the genus and also listed the parasites under their snake and lizard hosts from palearctic, ethiopean and oriental regions. *O. tandani* n. sp. can be compared with the following species: *O. elaphis* Harwood, 1932; *O. fibrata* Meggitt, 1927; and *O. ameivae* (Beddard, 1914) Baer, 1924. The present form differs from *O. elaphis* in the size of the strobila, size of suckers in relation to size of scolex, size of cirrus pouch, and size of onchospheres. From *O. fibrata* it differs in the width of the proglottids, size and extent of cirrus pouch, greater number of testes, and in possessing irregularly alternating genital pores. From *O. ameivae* it differs in the width of the strobila, size of scolex and suckers, size of cirrus pouch, and size of onchospheres.

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RESEARCH NOTES

HELMINTHS IN *QUAIL (COTURNIX COTURNIX)* IN TURKEY

During the autumn months of 1956, at the Laboratory of Veterinary Parasitology, Etlik, Ankara, 40 quail were examined for helminth parasites. Nearly all these birds were hunted in Province of Samsun, one of the poultry breeding centers near the Black Sea coast.

All the internal organs were removed, opened separately and examined macroscopically for adult parasites which might be present. The walls and contents of the crop, proventriculus, gizzard, small intestine, ceca and rectum were studied with the dissecting microscope, to determine the presence of parasites such as *Tetrameres* sp., *Heterakis gallinae* and others. Several smears were made and the scrapings and contents of the duodenum and of the cecum were studied with the compound microscope to determine the presence of ova, larvae and microscopic parasites. The liver, urinary and reproductive system were always examined for flukes.

Of 40 quail examined, helminth parasites were present in 26. Of these 2 were found to harbor only one species; 20 were hosts for two species and 4 were found to harbor three species. The highest prevalence of infection was by *Heterakis gallinae* (Gmelin, 1790) 92.2%, followed by*Raillietina echinobothrida* (Megnin, 1881) 76.9%, *Choanotaenia infundibulum* (Bloch, 1779) 15.4%, *Subulura differens* (Sonsino, 1890) 15.4%, *Tetrameres fissispina* (Diesing, 1861) 7.6%. —H. KURTPINAR, *Institute of Bacteriology, Laboratory of Parasitology, Etlik, Ankara, Turkey.*

THE OCCURRENCE OF *PANOPISTUS PRICEI* IN ILLINOIS

Panopistus pricei Sinitsin, 1931, was recovered from the intestines of 10 of 13 *Blarina brevicauda* (Say), the short-tailed shrew. The animals were trapped in Trelease Woods, a study area used by the Departments of Botany and Zoology at the University of Illinois, Urbana, Illinois. One to 22 trematodes were recovered per infected shrew. This fluke has been previously reported from Virginia by Sinitsin (1931) Beltsville, Maryland by Krull (1934, 1935), from Cazenovia, New York, by Krull and Mapes (1952), and from Ann Arbor, Michigan by Villella (1954).—NORMAN L. LEVIN, *Zoology Laboratory of the University of Illinois.*

RESEARCH NOTES

A BLOOD PARASITE FROM THE BAT, *TADARIDA BRASILIENSIS MEXICANA*

During a survey of blood parasites of Chiroptera from the San Antonio, Texas area, parasites of undetermined nature were found in the erythrocytes of 22 out of 196 *Tadarida brasiliensis mexicana.*

Thirty-one of the bats were examined immediately after capture, while the remainder were examined after they died in captivity (within 6 weeks after capture). Dead bats not immediately examined were refrigerated at −5° to 0° C from 1 hour to 5 days prior to examination. The time which elapsed between death and refrigeration varied from ½ hour to 14 hours.

Thick and thin smears were made from all bats; the blood was taken directly from the heart and was fairly easily obtained even from those bats which had been refrigerated. Smears were also made from the brain, liver, spleen, heart, and occasionally from the lungs, bone marrow, and peritoneal fluid. Hearts and intestines were fixed in 7.5% formalin and sectioned. All blood and tissue smears were stained with Giemsa, while the tissue sections were stained with hematoxylin-eosin. The parasites, however, were observed only in the blood smears.

Parasites were found in 7 of the 31 bats that were examined immediately, and in 15 of the remaining 165 bats which had been refrigerated. There were no observable differences in appearance or numbers of parasites from the bats examined immediately and those which were refrigerated.

In thick smears the organisms occurred in pairs and appeared cigar-, almond-, and dumbbell-shaped (Fig. 1); only rarely were single specimens noted. The size range in microns, of 100 specimens was 6.16–10.01 in length × 1.54–3.08 in width across the nucleus. The average size was 7.76 × 2.11.

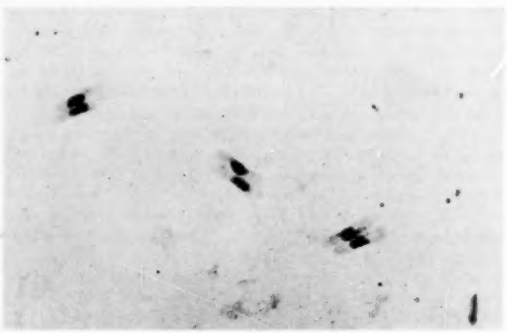


FIGURE 1. Parasites in a thick blood smear, × 960.

In thin smears the organisms were in pairs inside red blood cells, and appeared crescentric or almond-shaped. The size range, in microns, of 36 specimens was 7.70-9.24 in length × 1.93–3.08 in width across the nucleus. The average size was 7.97 × 2.72. The parasites were detected in thin smears from only 4 of the 22 infected bats. Between 2 and 30 infected red blood cells were found in these smears.

The nucleus appeared as a broad band stretched across the center of the organism and occupied approximately 1/4 to 1/3 of the body area; it usually contained 1 or more deeply stained eosinophilic granules. The cytoplasm stained pale blue and in rare instances seemed highly vacuolar; it too contained 1 or more eosinophilic granules.

A search of the literature failed to reveal any reports of similar parasites having been found previously in Chiroptera.

Blood slides were forwarded to Dr. R. D. Manwell, who stated that the organisms looked very much like hemogregarines.

It will be necessary for life-cycle studies to be conducted before these parasites can be classified as hemogregarines or otherwise. Until then the authors believe that the parasite should be considered as being of undetermined nature.

Acknowledgement is given to Sp3 Knapp for capturing the bats; to Dr. Manwell for examining the smears; and to the Brooke Army Hospital Photography Section for the photomicrograph.—ELLIOTT LESSER,* 1ST. LT., MSC; KENNETH F. BURNS, LT. COL., VC; AND CHARLES J. FARINACCI, COL., MC; *Fourth Army Area Medical Laboratory, Fort Sam Houston, Texas* (* Present address: *Regional Animal Diseases Laboratory, USDA, P. O. Box 952, Auburn, Alabama*).

NEW RECORDS OF MALLOPHAGA FROM WILD CHICKENS

The few published records of Mallophaga from wild chickens are based on specimens obtained from museum skins. Reported herewith are the results of several field collections made in Southeast Asia. Seven collections were made by Harry Hoogstraal in the Philippines; the skins of these hosts are now in the Chicago Natural History Museum. One collection in Laos and 27 collections in Thailand were made by H. G. Deignan, R. E. Elbel, and Boonsong Lekagul; the skins of these hosts are now in the U. S. National Museum. The collections in Laos and Thailand were made possible through the assistance of the U. S. Operations Mission to Thailand, and the U. S. National Museum.

The number of species of Mallophaga being reported is less than that normally found on domestic chickens. This is to be expected, since several of the species found on domestic chickens appear to have been derived from other hosts. Synonomy, illustrations, and distribution on domestic hosts, for each species, has been recently discussed by Emerson (1956. J. Kansas Ent. Soc. **29**: 63–79, plates I–XI).

Goniocotes gallinae (DeGeer), 1778.

PHILIPPINES. From *Gallus gallus gallus* (Linnaeus), 1758: 1 male collected at San Pedro, Culión, Calamianes.
THAILAND. From *Gallus gallus spadiceus* (Bonnaterre), 1792: 8 females collected from 4 specimens on Phu Lom Lo Mt., Kok Sathon, Dan Sai, Loei; 1 female collected at Ban Hua Thanon, Khlong Khlung, Kamphaeng Phet; and 2 males, 11 females collected on Phu Phak Khi Nak Mt., Kok Sathon, Dan Sai, Loei.
LAOS. From *Gallus gallus spadiceus* (Bonnaterre): 1 female collected at Ban Ton Phung (opposite Chiang Saen Kao, Thailand).

Goniodes dissimilis Denny, 1842.

PHILIPPINES. From *Gallus gallus gallus* (Linnaeus): 3 males, 4 females collected from 2 specimens at San Pedro, Culión, Calamaines.
THAILAND. From *Gallus gallus gallus* (Linnaeus): 6 males, 6 females collected from 2 specimens at Ban Sang Kho, Khok Phu, Sakon Nakhon; 4 males, 10 females collected from 2 specimens on Phu Kho Mt., Kan Luang, Na Kae, Nakhon Phanom. From *Gallus gallus spadiceus* (Bonnaterre): 5 males, 5 females collected from 3 specimens on Phu Phak Khi Nak Mt., Kok Sathon, Dan Sai, Loei; 16 males, 36 females collected from 10 specimens on Phu Lom Lo Mt., Kok Sathon, Dan Sai, Loei; 3 males collected at Hin Laem, Tha Khanum, Kanchanaburi; 1 female collected at Khao Suan Kwang, Nam Phong, Khon Kaen; and 1 female collected at Ban Muang Khai, Tha Li, Loei.
LAOS. From *Gallus gallus spadiceus* (Bonnaterre): 1 female collected at Ban Ton Phung (opposite Chiang Saen Kao, Thailand).

Lipeurus caponis (Linnaeus), 1758.

PHILIPPINES. From *Gallus gallus gallus* (Linnaeus): 1 male collected on McKinley Mt., Dávao, Mindanao; 4 males, 12 females collected at Carmelita, Busuanga, Calamianes; and 23 males, 20 females collected from 4 specimens at San Pedro, Culión, Calamianes.
THAILAND. From *Gallus gallus gallus* (Linnaeus): 4 males, 4 females collected at Ban Sang Kho, Khok Phu, Sakon Nakhon; and 10 males, 3 females collected from 2 specimens on Phu Kho Mt., Kan Luang, Na Kae, Nakhon Phanom. From *Gallus gallus spadiceus* (Bonnaterre): 10 males, 24 females collected from 3 specimens on Phu Phak Khi Nak Mt., Kok Sathon, Dan Sai, Loei; 22 males, 28 females collected from 11 specimens on Phu Lom Lo Mt., Kok Sathon, Dan Sai, Loei; 1 female collected at Hin Laem, Tha Khanun, Kanchanaburi; 5 males, 3 females collected at Khao Suan Kwang, Nam Phong, Khon Kaen; and 1 male collected at Si Than, Wang Saphung, Loei.
LAOS. From *gallus gallus spadiceus* (Bonnaterre): 1 male, 5 females collected at Ban Ton Phung (opposite Chiang Saen Kao, Thailand).

Menacanthus pallidulus (Neumann), 1912.

PHILIPPINES. From *Gallus gallus gallus* (Linnaeus): 1 male, 1 female collected at San Pedro, Culión, Calamianes.
THAILAND. From *Gallus gallus spadiceus* (Bonnaterre): 2 males, 2 females collected at Khao Suan Kwang, Nam Phong, Khon Kaen; 2 males, 2 females collected at Ban Khlua Klang, Prachuap Khiri Khan; and 1 male, 1 female collected on Phu Lom Lo Mt., Kok Sathon, Dan Sai, Loei.

Menopon gallinae (Linnaeus), 1758.

PHILIPPINES. From *Gallus gallus gallus* (Linnaeus): 21 males, 12 females collected on McKinley Mt., Dávao, Mindanao; 33 males, 20 females collected from 4 specimens at San Pedro, Culión, Calamianes; 6 males, 13 females collected at Carmelita, Busuanga, Calamianes; and 22 males, 9 females, collected at Dimaniang, Busuanga, Calamianes.
THAILAND. From *Gallus gallus gallus* (Linnaeus): 5 males, 3 females collected from 2 specimens at Ban Sang Kho, Khok Phu, Sakon Nakhon; and 1 male, 2 females collected on Phu Kho Mt., Kan Luang, Na Kae, Nakhon Phanom. From *Gallus gallus spadiceus* (Bonnaterre): 13 males, 10 females collected from 3 specimens on Phu Phak Khi Nak Mt., Kok Sathon, Dan Sai, Loei; 42 males, 27 females collected from 8 specimens on Phu Lom Lo Mt., Kok Sathon, Dan Sai, Loei; 1 female collected at Ban Khlua Klang, Prachuap Khiri Khan; 1 male, 1 female collected at Ban Hua Thanon, Khlong Khlung, Kamphaeng Phet; 3 females collected at Khao Suan Kwang, Nam Phong, Khon Kaen; 1 male collected at Ban Muang Khai, Tha Li, Loei; 3 males, 1 female collected at Si Than, Wang Saphung, Loei; and 3 males collected at Ban Na Muang, Na Haeo, Dan Sai, Loei.
LAOS. From *Gallus gallus spadiceus* (Bonnaterre): 3 males, 2 females collected at Ban Ton Phung (opposite Chiang Saen Kao, Thailand).

Oxylipeurus dentatus (Sugimoto), 1934.

THAILAND. From *Gallus gallus spadiceus* (Bonnaterre): 35 males, 40 females collected from 7 specimens on Phu Lom Lo Mt., Kok Sathon, Dan Sai, Loei; 7 males, 7 females collected at Ban Hua Thanon, Khlong Khlung, Kamphaeng Phet; 4 males, 10 females collected from 3 specimens on Phu Phak Khi Nak Mt., Kok Sathon, Dan Sai, Loei; 3 males, 6 females collected at Ban Muang Khai, Tha Li, Loei; and 2 males, 1 female collected at Si Than, Wang Saphung, Loei.—K. C. EMERSON, *Stillwater, Oklahoma* AND ROBERT E. ELBEL, *Lawrence, Kansas.*

RESEARCH NOTES

THE ECOLOGY OF MARINE DERMATITIS-PRODUCING SCHISTOSOMES. II. EFFECTS OF CERTAIN ENVIRONMENTAL FACTORS ON EMERGENCE OF CERCARIAE OF *AUSTROBILHARZIA VARIGLANDIS*

As part of a general investigation of the ecology of marine dermatitis-producing schistosome larvae in northern New England (Sindermann and Gibbs 1953, Maine Dept. Sea and Shore Fish. Res. Bull. No. 12: 1–20), examination of emergence of cercariae of *Austrobilharzia variglandis* under varying environmental conditions has been made. Temperature, salinity, and nutritional state of the snail host have been related to changes in emergence.

Nassarius obsoletus (*Nassa obsoleta*), molluscan intermediate host of the avian schistosome *Austrobilharzia variglandis*, has been maintained in graded salinities at constant temperature (20° C) and the percentage of infected snails emitting cercariae, as well as the number of cercariae emerging at each salinity, recorded daily. The greatest numbers of cercariae emerged and the greatest percentages of infected snails emitted cercariae at salinities between 15 and 25 parts per thousand, with marked reduction at higher and lower salinities. Respiration studies of both infected and uninfected snails in the same graded salinities indicated greater oxygen consumption at salinities between 15 and 25 parts per thousand than at higher or lower figures, suggesting that cercarial emergence may be related to snail metabolism. This is further suggested by the fact that starved infected snails held at normal inshore salinity (32 parts per thousand) and constant temperature displayed a 3- to 4-day interval between peaks of cercarial emergence, which might possibly be associated with rhythmic release of host digestive enzymes. Feeding of starved snails resulted in massive cercarial emergence within 48 hours.

Cercarial emergence from infected snails held in aquaria with seasonal light and sea water temperatures declined with reduction in water temperature in autumn and ceased altogether at 6–8° C, to resume again the following spring when this water temperature was again reached. Cercarial emergence from snails removed from these conditions could be stimulated experimentally at any time during the winter by raising the water temperature above 10° C. Infected snails maintained at seasonal sea water temperatures and a 16-hour day length throughout the winter followed the same pattern of cercarial emergence as those exposed to normal day lengths.

Relating these observations to the intertidal mud flat habitat, the migratory activities, and the seasonal changes in infection of the snail host (Sindermann 1956, J. Parasit. **42** (Suppl): 27) it becomes possible to predict periods of heaviest cercarial emergence and potential human danger in areas where infected *Nassarius* are found.

This investigation was supported in part by research grant E-974 from the National Microbiological Institute, National Institutes of Health, Public Health Service.—C. SINDERMANN, A. ROSENFIELD, AND L. STROM, *Fisheries Research Station, Boothbay Harbor, Maine.*

A RAPID SLIDE HEMAGGLUTINATION TEST FOR THE DETECTION OF ANTIBODIES TO *TRICHINELLA SPIRALIS*

Boyden's technique (1951, J. Exp. Med. **43:** 107-120) of adsorbing protein-antigen onto tanned red cells has been used recently by some investigators as a possible means of detecting antibodies against parasitic infections. By this method, Kagan (1955, Science **122:** 376–377) was able to detect antibodies in sera of rabbits immunized with *Schistosoma mansoni* and *Schistosoma douthitti.* Kagan and Bargai (1956, J. Parasitol. **42:** 237–245) described similar findings in rabbits and rats infected with *Trichinella spiralis.* In a preliminary study, Jacobs and Lunde (1957, J. Parasitol. **43:** 308–314) detected antibodies from toxoplasmosis patients by the hemagglutination technique. Having used for several years the bentonite flocculation test (Bozicevich *et al,* 1951, Pub. Health Rpts. **66:** 806–814; Sadun, E. H., and Norman, L., 1955 Pub. Health Lab. **13:** 147–152) in the detection of antibodies to several helminthic infections, the authors investigated the possibility of substituting in this test tanned erythrocytes for the bentonite particles. The present study is concerned primarily with the development of a rapid slide hemagglutination test as a serological procedure for titrating sera for antibodies against *Trichinella* infections.

An acid soluble protein fraction of *Trichinella* larvae was used as antigen (Melcher, 1943, J. Infect. Dis. **73:** 31–39). Sensitized sheep erythrocytes were prepared in strict adherence to Boyden's method. Twofold serial dilutions were made of the human sera to be tested with 1 : 100 normal rabbit serum in buffered saline starting at 1 : 5. All the sera were inactivated. The normal rabbit serum was previously absorbed with an equal volume of packed red blood cells. Waxed rings were prepared on clean glass slides and 0.1 ml of each serum dilution was placed within each ring. With a specially prepared pipette delivering between 60 to 80 drops per mililiter, one drop of the sensitized erythrocytes was added to the center of each waxed ring containing the diluted serum. The slide was placed on a rotating apparatus and rotated for 15 minutes. The readings were done and interpreted as with the bentonite flocculation test. Normal sheep cell control, tannic acid control and normal serum control were carried out with each test.

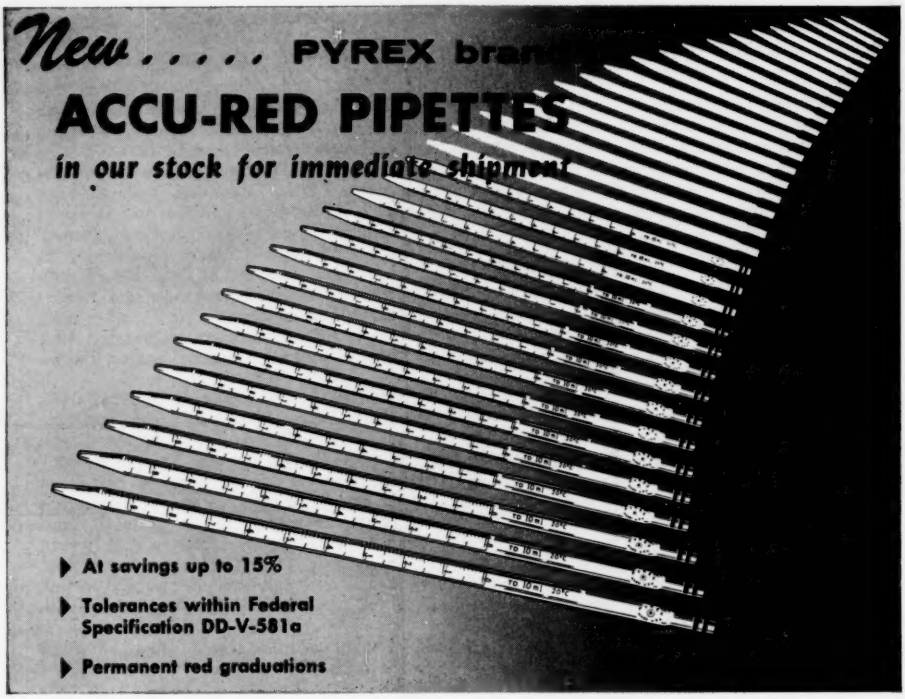
A total of 43 human sera were tested. Of these, 14 were selected as coming from individuals with a proved or highly suggestive trichinosis, on the basis of detailed clinical histories and laboratory tests such as muscular biopsy and serological tests. Twenty-nine normal sera were selected from individuals with no indication of trichinosis. All of the sera of the first group gave positive reactions and all of the sera of the second group gave negative reactions. These results were in complete agreement with those obtained by testing the sera with the bentonite flocculation test. In order to gain some indication as to the ability to obtain consistent results with the slide hemagglutination test, 5 human sera were tested repeatedly with different lots of sensitized erythrocytes. The results were compared with those obtained with the bentonite flocculation test. As indicated in Table I, the results were consistent even though variations

TABLE I.—*Slide hemagglutination reactions with different lots of sensitized cells*

Serum number	Number of lots tested	Slide hemagglutination test		Reciprocal of flocculation titer
		Number of pos. reactions	Reciprocal of titers	
1	5	5	20 ; 80 ; 160 ; 160 ; 640	160
2	7	7	20 ; 20 ; 40 ; 40 ; 40 ; 80 ; 80	40
3	6	6	20 ; 20 ; 80 ; 160 ; 320 ; 320	320
4	7	7	5 ; 20 ; 20 ; 40 ; 40 ; 40 ; 80	10
5	6	5	Neg ; 10 ; 10 ; 20 ;20 ; 80	10

in titers occurred with different batches of antigen.

These preliminary experiments indicate that antibodies in the sera of trichinosis patients with *Trichinella* infections can be measured in a slide hemagglutination test by adsorbing the antigen onto tanned erythrocytes. Since no attempts have been made to evaluate this test and to compare it with the standard hemagglutination test, no knowledge is yet available as to the sensitivity and specificity of this test. It should be pointed out, however, that this test offers the great advantages of being performed in a very short time, of being much easier to read, and of requiring very minute amounts of antigens and antisera.—E. H. SADUN AND D. S. ALLAIN, *Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Atlanta, Georgia.*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